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The Journal of Laboratory and Clinical Medicine

VOL. III. ST. LOUIS, MARCH, 1918 NO. 6

ORIGINAL ARTICLES

SUGAR METABOLISM AND DIABETES*

BY HUGH MCGUIGAN, M.D., CHICAGO, ILL.

THE most interesting thing in medicine is to watch its growth. When a disease is fully understood, its cause known, and its treatment definite, there is little of interest in it. If a patient has malaria, hookworm, or diphtheria, there remains only the routine treatment with quinine, thymol, chenopodium, or antitoxin. The history of the disease and the development of the treatment still retains something of interest, but it lacks the fascination of the research stage. Apart from the purely interesting phase in the development of a disease, the proper perspective can be obtained only by a study of its history and development.

Osler has said that if a person has a knowledge of tuberculosis as developed from the time of Koch, he may have a comparatively good knowledge of the subject, but if he knows nothing of the disease before that time, he still has much to learn about it. This statement places a fair estimate on the study of medical history, and if it be true of tuberculosis, it is doubly true of diabetes. Like tuberculosis, diabetes has been known from the earliest times. Aretæus[1] in the first century left a classic record of the symptoms. Susruta,[2] about the seventh century, knew of the peculiar odor and sweet taste of diabetic urine. Apart from the taste and smell, little more was known until the sixteenth century when Paracelsus[3] (1493-1541), the founder of chemical pharmacology and therapeutics, evaporated a liter (measure) of diabetic urine and found that it contained four ounces of what he called salt, or more than four times the amount found in normal urine. From this discovery, Paracelsus taught that the blood of diabetics contained a salt, which, when eliminated by the kidneys, caused the polyuria. Since the salt in the blood, according to him, was

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the cause of the disease, he taught that the treatment should be directed not against the kidney, but toward the cause further back. The kidney, however, had been considered the seat of the disease since the time of Galen,[4] and but little attention was paid to the opinion or teaching of Paracelsus. Whatever we may think of Paracelsus, he, at least, made men think, and better still, made them doubt the traditions and beliefs in medicine that were not founded on fact. It makes little difference what we may think of a man, if he presents facts that can be substantiated, we can not get away from them. If diabetic urine contains four times as much solid matter as normal urine, the fact stands, and must be accepted.

In 1674 Thomas Willis[5] emphasized more strongly than his predecessors the sweet taste of diabetic urine, and Dobson,[6] in 1774, like Paracelsus, evaporated diabetic urine and concluded that the residue was analogous to sugar. Dobson is generally credited with the discovery that diabetic urine contains sugar, but this credit has been protested and he has even been accused of plagiarism on this point. There is no doubt but that at the time others reached the same conclusion. This work of Dobson marks the beginning of the modern era of carbohydrate metabolism and the subject is so closely related to diabetes that the two can not be separated. About the same time, Lavoisier[7] proved that the results of the oxidation of foodstuffs are the same within the body as outside of it and that the body heat is derived from the oxidation of the body substance. In 1815 the French chemist Chevreul[8] demonstrated the identity of grape sugar and diabetic sugar. About this time also Dobson[9] noted that the serum of some diabetics had a sweet taste, and Claude Bernard seventy years later (1847) proved definitely that the blood of diabetics contains sugar.[10] Before this time, however, Tiedemann and Gmelin[11] (1821) discovered that during the digestion of starches in the body, glucose is formed and from this fact they inferred that glucose must be an ingredient of the blood. This inference was confirmed by Magendie and Frerichs in the early forties, several years before Bernard began work, but the work of Bernard on this point is more convincing, and is generally accepted as the first definite proof. Bernard showed the origin of the blood sugar and definitely connected diabetes and carbohydrate metabolism.[12] The term "metabolism" was first used by Liebig[13] in 1842 and is used to include the total chemical changes of foodstuffs under the influence of the enzymes and living cells of the body.

Since we have defined metabolism and almost every one knows, or thinks he knows, what diabetes is, for completeness we might define sugar also. The term itself means something with a sweet taste, and with this as the most characteristic quality, the method of analysis and detection was rather easy in the early days. Unfortunately for this rapid method, it has been discovered that all things with a sweet taste are not sugars, for bodies like glycerin, glycol, saccharin and even lead acetate also have a sweet taste. It is, therefore, quite apparent why some of the early reports of sugar in the blood were not definitely settled until a much later period. Many textbooks on chemistry, while they tell the characters and reactions of sugar, hesitate to give a rigid definition. Time may change the scope of its definition like that of many other chemical bodies. However, for the present, it may be said that a sugar is a carbo-

hydrate that contains one carbonyl group and several hydroxyl groups. One of the hydroxyl groups must be linked directly to a carbon atom in union with the carbonyl group, so that the characteristic group of these compounds is: CHOH-CO.[14]

The role of Claude Bernard in the field of sugar metabolism is especially prominent. While he was an intern in the Paris hospitals under Magendie,[15] the therapy of Broussais[16] and Rasori[17] was still in vogue, and although Magendie taught against it, bleeding for almost every disease was a routine treatment. Liters of blood drawn for the therapeutic effect were available to any investigator. Thus it is quite comprehensible why Bernard's thesis for the doctorate was an investigation of the blood sugar. Although Bernard is credited with the discovery of sugar in the normal blood, in his thesis for the doctorate he contended that normal blood did not contain a trace of sugar.[18] It was only after a controversy with the chemist Figuier[19] that he finally proved that sugar is a constituent of normal blood.

His failure at first to detect sugar, when satisfactorily explained, led him directly to another important discovery, that is, the phenomenon of glycolysis or the disappearance of the sugar from the blood on standing. In his first work he used blood that had stood in the hospital wards from twelve to twenty-four hours. When he worked with freshly drawn blood and found that it always contained sugar, he was quick to infer that the sugar in the old blood had disappeared on standing. Investigation easily proved the truth of his conjecture. This phenomenon is of interest in view of the later work of Cohnheim and others on the mechanism of sugar oxidation in the body, and was the foundation of still further research by Bernard.

When the presence of sugar in the normal blood had been established and also the fact that sugar is formed from starches in the gastrointestinal tract, it was quite apparent that the sugar in the blood may be derived directly from the carbohydrates of the food. There still remained to be determined: (1) the changes, if any, that the sugar undergoes during its passage through the intestinal wall; (2) its condition in the blood; (3) the changes through which it passes before oxidation in the body; (4) the difference, if any, between diabetic and normal blood. It is believed by some that the blood sugar is in a combined state or united with something of the nature of an amboceptor and that this amboceptor is lacking in diabetes. This, and other theories have been advanced to explain the increase in the blood sugar in diabetes and the passage of the sugar into the urine.

The most important and fundamental work in sugar metabolism was the discovery of glycogen by Claude Bernard (1857). After many attempts to produce diabetes artificially, he found that puncture or injury of the fourth ventricle caused glycosuria. He then attempted to find the source of the sugar which appeared in the urine, and soon discovered that arterial blood contained more sugar than the venous. When the carotid artery contained 0.12 per cent, the jugular vein contained 0.08 per cent. The relation of the femoral vein and artery was similar. It was evident, therefore, that sugar is lost from the blood to the tissues during the circulation.

With a flexible rubber catheter or sound, he was able to make soundings

and to take blood for analysis from almost any point of the venous circuit.[22] He introduced the sound into the jugular vein, pushed it down into the right heart and found that the venous blood in this region contained as much sugar as the arterial blood. It was clear that somewhere between the femoral vein and the right heart, sugar was added to the blood. To definitely locate the source of this addition, he passed the sound through the heart and down to the level of the kidneys and found that the venous blood at that point contained 0.08 per cent sugar. The sound was then carefully withdrawn to the level of the hepatic vein, where the blood was found to contain 0.14 per cent sugar. In another experiment, he introduced the sound into the crural vein to the level of the hepatic vein and with a syringe, aspirated the blood for analysis. It contained 0.266 per cent, while the inferior vena cava contained 0.088 per cent. By these, and other similar experiments, he proved that the liver is the source of the added sugar.

He also found that if the fresh liver was quickly removed from an animal and thrown into boiling water, only small amounts of sugar were obtained. But there went into solution in the water a substance which made an opalescent solution and which, when boiled with acid, or acted upon by saliva, yielded a reducing sugar. Bernard[23] was also able to isolate and study this substance, which he called *glycogen*. The increased discharge of this substance from the liver is believed to be the commonest cause of most forms of hyperglycemia—at least of those that are transitory. Bernard thought that the increased blood flow through the liver was chiefly responsible for the hyperglycemia, by bringing about an increased rate of the hydrolysis of the glycogen, or glycogenolysis. Others have thought it is due to changes in glycogen formation from sugar, or glycogenesis.

Since the condition of the glycogen in the liver seems to be immediately responsible for the hyperglycemia, it is important to know whether the increased glycogenolysis or decreased glycogenesis is due directly to the liver itself, or whether it is due to defects in other organs which act indirectly on the liver. Almost all other organs have been incriminated in the trouble.

Nicolas and Gueudville, following Rollo,[24] regarded diabetes as a derangement of the digestive functions with the initial lesion in the intestine, and perhaps due to changes in the gastric juice. Although they founded a system of treatment based on this theory, they never attempted to support it by experimental evidence. Recently, Croftan[25] thought that he obtained evidence showing that the intestine is necessary for the polymerization of sugar and the formation of glycogen. According to him the sugar must pass through the intestinal wall, otherwise no glycogen can be formed. He claimed that if the sugar be injected into the veins going directly to the liver, without first passing through the intestine, no glycogen is formed. Pflüger[26] denies Croftan's contention absolutely, and Macleod[27] rejects his evidence as entirely inadequate.

There is also much circumstantial evidence against the intestines being important in the glycogen formation. Karl Grube[28] showed that the liver polymerizes only the monosaccharides. Again, if any of the polymerized forms of sugars, dextrines, or starch are injected into the blood, the diastases of the blood immediately break them down into simple sugars. What is not hydrolyzed

is excreted by the urine or intestine. It would seem, however, that if any polymerization of the sugar from the intestinal tract takes place while passing through the gut wall, it would be hydrolyzed again in the blood, and energy lost in such a mechanism. It is still possible that some other modification of the sugar takes place to aid in glycogen formation, but more evidence is necessary before it deserves serious consideration.

Against this assumption is the condition of the sugar in the blood. The weight of evidence now favors the opinion that the sugar in the blood exists in the same state as it exists in a water solution. The proof of this is as follows: Rona and Michaelis[29] have shown that the proteins of the blood can be precipitated by colloidal ferric hydroxide, and when this is done, all of the sugar is in the filtrate. By this method, the minimal amount of change in the proteins occurs, and suggests strongly that the blood sugar is uncombined. Many

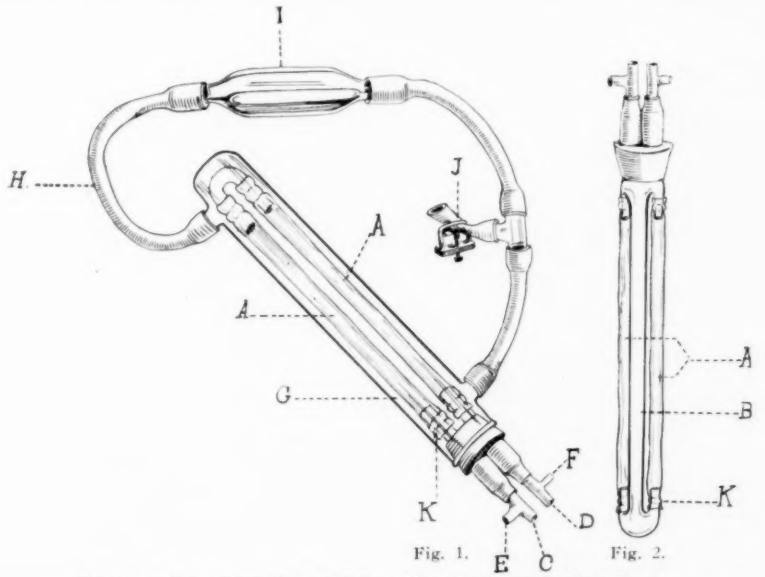

Fig. 1. Fig. 2.

Figs. 1 and 2.—Dialyzing Apparatus Not Requiring Anticoagulants.

1.—Complete apparatus.
2.—Interior part of apparatus.
A—Collodion tubes.
B—Glass support or frame.
C and *D*—Glass cannula, *C* is inserted into the artery; *D*, into the corresponding vein. The side tubes, *E* and *F*, are closed with a screw clamp, and rubber, and permit a flushing out of the apparatus without disconnecting, in case of clotting.

When the artery is opened, the blood circulates through the collodion tube *A*, and is returned to the animal into the vein by *D*. Water, or saline, surrounds the tubes in the jacket *G*.

The rubber tubing *H* and the reservoir *I*, when raised and lowered, cause the fluid around the tubes to move or circulate, and thus hasten dialysis. The screw clamp, *J*, permits one to regulate the pressure within the jacket and around the collodion tubes. This is an important procedure and the pressure should be so regulated that the pulsations in the tube resemble those in the artery.

Special precautions should be taken to insure against leaking at the glass and collodion joints *K*. To secure this, we used adhesive tape and thread ligatures.

others have dialyzed the freshly drawn blood and have been able to dialyze out all of the sugar. If this were a colloidal form, such a separation would be improbable. Even such mild manipulations may break up weak combinations of the sugar with proteins or other colloids, and so to get still nearer the actual condition, C. L. Hess[30] and I dialyzed the normal circulating blood and found that all of the sugar can be dialyzed out. The apparatus which we used was a modification of the dialyzing apparatus used by Abel and his coworkers,[31]

and consists essentially in placing a segment of an artificial artery, composed of collodion, between the cut ends of a normal artery in such a way that it can be immersed in water or saline, and dialysis accomplished while the blood is circulating within the animal. The general technic and the illustration of the instrument will suffice here. Details are given in the *Journal of Pharmacology*, 1914, vi, 45.

The general technic of the experiments was as follows:

Dogs were anesthetized with different anesthetics, and the apparatus attached to the carotid artery and external jugular vein, on the same side, or in the femoral vessels. By avoiding all roughness in the glass and by keeping a pulsation in the dialyzer, we were able to work without an anticoagulant, and by raising and lowering *I*, to cause a circulation of the liquid around the dialyzing tubes, which greatly hastened dialysis. The results obtained by this method are illustrated in Table I.

TABLE I

ANESTHETICS USED	DIALYSIS	*Percentage of sugar in* PLASMA	DIALYSATE	PLASMA H_2O	DIALYSATE H_2O
Morphine	3.0 hrs.	0.135	0.139	0.147	0.141
Morphine	5.0 "	0.125	0.133	0.136	0.135
Morphine	6.0 "	0.088	0.099	0.096	0.100
Ether	4.5 "	0.083	0.087	0.090	0.088
Morphine and ether	5.0 "	0.073	0.082	0.080	0.083
Urethane	2.0 "	0.151	0.168	0.164	0.169
Urethane and ether	3.5 "	0.163	0.175	0.177	0.177
Urethane and ether	4.5 "	0.153	0.169	0.167	0.171
Urethane and ether	1.0 "	0.157	0.173	0.170	0.175

While this work shows that the sugar of the blood is in the same state as in a water solution, there still remains the possibility of a loose combination similar to oxygen and hemoglobin, which might be broken by even such mild manipulations as dialysis. It is difficult to imagine a more delicate method than the dialysis of the normal circulating blood. However, we have tried every method that has suggested a possibility of aiding in the solution of the problem. In comparing different methods of analysis of blood sugar, we found a great variation in the results. It was hard to get a perfect quantitative agreement when different methods were used. Recently some investigators working on the normal level of the blood sugar have come to the conclusion that the higher levels reported are due to excitement. It has been known from the time of Bernard that nervous influences[32, 33] play a great part in regulating the amount of sugar in the blood and that there is some change in the amount of the blood sugar during excitement. We have found that the greatest cause for the variation is in the methods of analysis and especially in the composition of Fehling's solution. The composition of this solution as recommended by various workers varies greatly in alkalinity and we have found that this is the chief cause for the variation reported in the normal level of the blood sugar.[40]

As an indication of the variation in alkalinity of Fehling's solution, the U. S. Pharmacopeia VII recommended 125 grams KOH in 500 c.c. Revision

VIII reduced this to 75 and the current issue IX reduces this to 50 grams. While this difference in alkalinity makes relatively little difference in the yield of sugar from a pure water solution, it makes a great difference in blood analysis. The reason seems to be that the precipitants used to remove proteins leave an interfering body in varying amounts in the filtrate; and in presence of stronger alkali, this body, whatever it may be, prevents the reduction or precipitation of the cuprous oxide.

The two most used methods for the determination of sugar in the blood are the Bertrand[34] modification of Fehling's method and the Lewis-Benedict method.[35] In a recent investigation on the condition of the sugar in the blood, E. L. Ross and I used both of these methods.

Benedict uses picric acid for the removal of the proteins, and the protein-free picric acid filtrate is simply heated with sodium carbonate. In the presence of sugar the picric acid is reduced to picramic acid, which gives a red color. The depth of the color depends on the amount of sugar present, so that the amount can be determined by comparing the color with that of a known solution of sugar.

While engaged in this work, we first noticed the note by W. B. Smith[36] that picric acid does not interfere with the results of Fehling's solution. Such being the case, it renders the two methods directly comparable since the conditions of precipitation can be made exactly alike. The difference, however, between the analyses of the blood-picric filtrate by the two methods was so enormous that we doubted the truth of the statement. We found that in control work, picric acid does not interfere with the Bertrand method, as the following results show: A solution of dextrose was prepared, approximately 0.1 per cent *in water* and determined by the Bertrand method with the following results:

Normal .110%
.110%

After the addition of 1 gram picric, an amount greatly in excess of saturation, but which went into solution on boiling, the results were:

.113%
.115%

This slight increase might be considered negligible and within the limits of error. It is constant and due to a salt action, as salts to some extent increase the reduction of alkaline copper.

This influence of salt on the result of the Bertrand method is shown by the following figures:

Sugar in Water	*Sugar in Salt*			
I	II	III	IV	V
	2% Na_2SO_4	5% Na_2SO_4	12% Na_2SO_4	25% Na_2SO_4
0.85%	0.88%	0.92%	0.110%	0.110%

Beyond 12 per cent, the influence of the salt does not change. We quote this salt, and 10 to 20 per cent, because hitherto in the precipitation of the proteins from blood, we have used sodium sulphate and acetic acid to a great ex-

tent. The most probable explanation of the salt action is its influence on the boiling point of the solution.

Having convinced ourselves that picric acid does not interfere with the results of the Bertrand method, and that what slight influence it has, is to increase the yield of sugar, we investigated the differences in the results of the Bertrand and the Lewis-Benedict methods. The comparison here is very direct since we use picric acid to remove the proteins in each case, but any method of removal of the proteins of the blood, where acid is avoided, will give results similar to these quoted. We had corroborated the work by the use of (1) alcohol, (2) methyl alcohol, (3) colloidal iron, (4) sodium sulphate, (5) basic lead acetate, (6) dialysis of the serum.

The Lewis-Benedict method was carried out as recommended by Meyers and Bailey.[35] The following table shows some of the results we have obtained by these methods directly, and also by Bertrand's method after "hydrolysis." Details of the work are given in the *Journal of Biological Chemistry*, 1917, xxxi, 553.

	Bertrand Direct with 12.5% KOH, Fehling solution	Lewis-Benedict	Bertrand after "hydrolysis" or directly with 5 per cent KOH, Fehling solution
Average.....	0.024	0.094	0.099

In seeking for the cause of the great difference between the Bertrand and the Benedict methods, we naturally suspected the presence of a sugar combination—"virtual sugar"—or a sugar of the maltose type, and for this reason hydrolyzed and obtained a rise in the amount, and a figure which in most cases agrees very closely with the Benedict method. There were many facts, however, that made us doubt that there was an actual hydrolysis. First, to get the highest results, we had to use a certain amount of acid, which reduced the alkalinity of Fehling's solution from 12.5 per cent KOH to 5 per cent, and, second, the time necessary for the completion of the supposed hydrolysis was very short, and higher results were obtained if the acid used was not neutralized before applying the Fehling solution. Finally we found if the acid were added directly to the Fehling solution; i. e., lessened alkalinity of the Fehling, the same results were obtained as after the supposed hydrolysis. It is evident, therefore, that lessened alkalinity, and not hydrolysis, is the true explanation. The reason for not neutralizing after hydrolysis and before using the Fehling was due to the fact that there is great variability in the alkalinity of Fehling's solution, as recommended in standard works, and even without neutralizing, we had still a strongly alkaline liquid that corresponded to many so-called Fehling solutions.

With a water solution of dextrose, almost theoretic results can be obtained with Bertrand's method as we have used it. The above results of blood analysis are so striking, and so out of harmony with current opinion, that errors in method, or the presence of interfering bodies, must be seriously considered. Traces of protein or other bodies, which hold cuprous oxide in solution were thought of. The presence of such bodies at first seemed improbable, as is shown by the following work.

First, dextrose added to normal or to diabetic blood can all be recovered.

This has been shown in many cases. Second, the result of dialysis shows that whatever the nature of such a body may be, it is dialyzable. Dialysis does not preclude peptone bodies, which perhaps would be the protein that would interfere most. Such a body, however, would seem to be excluded by the lessening of the alkalinity which removes the effect, and could not remove the interfering body. While the presence of peptone thus seems improbable, its possibility still remains, for we know that traces of peptone beyond detection or recognition markedly influence the Bertrand method, while they have little influence on Benedict's method. If such a body is present its solvent effect is limited, because added sugar can be recovered and changes in the blood sugar are easily recognized. Third, the action of ether: In anesthetized animals, and in diabetics, we can detect no change in the blood protein. Here again the presence of a disturbing body might well escape us, because the amount of sugar in these cases is so large that a change which would make a large percentage change in normal blood would be relatively insignificant here.

Instead of a protein the interfering body might be a dextrin-like body, but the fact that it will dialyze and is easily "hydrolyzed" removes the probability of a dextrin.

Very recently Scott[37] has suggested the presence of a lecithin combination, but this seems improbable since extraction of the picric filtrate or of the blood with ether before precipitation does not change the sugar in any way. This does not disprove Scott's contention that lecithin may combine with dextrose, as he was working under different conditions. It is also not a glycogen-like body because when alcohol is used to precipitate the proteins, the glycogen would be removed, and yet we obtain results *similar* to the picric acid method.

The only investigator, so far as we know, who has reported the normal level of the blood sugar nearly as low as the figures we give for the direct determination after precipitation by picric acid is Shaffer.[38] His average for the normal blood sugar in four dogs was 0.036, 0.020, 0.046, and 0.026 per cent, figures that agree closely with ours by the direct picric acid method. His results after anesthesia show a considerable rise, showing also, we think, agreement with our explanation that sugar added by any cause can be recovered. The low results he at first obtained we think are due to the solvent action of the strong alkali on the cuprous oxide which is increased by a yet unknown body in the blood. This solvent action is fully satisfied by the amount of sugar in the normal blood so that added sugar or the increase due to anesthesia may be recovered. This solvent action is much greater in a blood filtrate than in a water solution. If the picric filtrate is boiled alone or if sodium sulphate is added and it is boiled, a higher sugar yield is obtained. This seems to be due to the removal of an interfering body.

Anesthesia and morphine increase the amount of sugar when determined by the Bertrand method after precipitation of the proteins by picric acid. This action is apparently not due to the breaking up of a sugar combination, because blood extracted directly with ether is not changed, nor is picric blood filtrate changed by similar treatment.

That morphine increases the sugar as determined by this method is shown by the following experiment.

	Fehling Solution	
	12.5% KOH	5% KOH
Normal dog. Blood sugar direct	0.015%	0.08%
3 hrs. after 0.15 gm. morphine	0.165%	0.225%
Normal dog	0.020%	0.110%
After ether	0.080%	0.156%

The opinion has been expressed by more than one writer that the sugar in the blood exists in combination with something of the nature of an amboceptor.[38] There are some facts brought out by the picric acid precipitation of proteins that might be used to sustain this theory. Since the use of picric acid seems to show that the blood sugar is not a simple solution of glucose in water, its use also permits the testing of the amboceptor hypothesis and to a considerable extent supports that theory.

For example, Shaffer[38] found that dextrose added to a solution of sugar that had fermented with yeast could not be completely recovered. We have been able to confirm this statement, and since the utilization of the dextrose by the yeast cell is probably by the same mechanism as in the animal cell, this masking or combining with the sugar, suggests a similar preliminary step in the animal body. Accordingly, we have tested blood by:

1. Adding sugar directly to it when fresh, and determining the amount of the added dextrose that could be recovered.
2. The same addition after blood had undergone glycolysis.
3. Comparing normal and diabetic blood.

In the normal blood the *added* sugar can be recovered, while in the blood that had been freed from sugar by glycolysis, added sugar is immediately masked as in yeast fermentation, and can not be recovered until after the addition of acid. This suggests that the blood sugar normally is in combination, and that when the sugar is removed by glycolysis, the amboceptor[39] still retains the power to unite with free dextrose, converting it into the same form as the dextrose normally existing in the blood.

In like manner we determined the sugar in *normal* blood directly by the picric acid Bertrand method, and found that all the added sugar can be recovered, while in blood that had undergone glycolysis, a certain amount apparently combined with the amboceptor, and could be recovered only after adding acid or reducing the alkalinity of Fehling's solution. The following experiments illustrate this.

Normal blood was glycolyzed until it showed no sugar when tested with Fehling's solution.[40] The Benedict method showed 0.016%; Fehling containing 2.5 per cent NaOH or 10 per cent Na_2CO_3 gave 0.01 per cent. If, now, 100 c.c. of this glycolyzed blood be precipitated with picric acid and to the picric acid filtrate we add 10 c.c. of dextrose solution—or 0.102 per cent—and determine how much of this can be recovered by varying the alkalinity of Fehling's solution, we find for the original undiluted Fehling containing 12.5 per cent NaOH—only 60 per cent recovered, or 0.060. Five per cent NaOH gives 0.110; 2.5 per cent gives 0.120 per cent. We thus find that added sugar can be recovered if not too much alkali is added, or if sodium carbonate is used instead of KOH.

Controls with water solution gave with 12.5 per cent NaOH, 0.102 per

cent; with 5 per cent NaOH, 0.110 per cent. With the weaker alkali, consequently, we recover the total sugar added, and the actual or total sugar of the blood.

The following results show how changing the alkalinity influences the yield of the sugar as determined by Bertrand's modification of Fehling's solution.

NaOH% in Fehling's sol.	Dog's blood sugar
12.5%	0.018%
5 %	0.090%
4 %	0.105%
2.5%	0.122%

The Lewis-Benedict method on this blood gave 0.106 per cent.

Increasing the amount of copper in the Fehling solution while the alkalinity remains constant (12.5% NaOH) has the same effect as decreasing the alkalinity and keeping the amount of copper sulphate constant, as the following analysis of dog's blood will show.

$CuSO_4$	Per cent sugar
2%	0.021%
4%	0.044%
8%	0.096%

A solution of dextrose in water or in picric acid solution is not nearly so much influenced as a blood filtrate by the variation of the copper, or by changes in the alkalinity. The following table shows the relatively slight variation of the same dextrose solution in water by varying the copper in Fehling's solution, while the alkalinity remains 12.5% NaOH.

$CuSO_4$	Per cent dextrose
2%	0.070%
4%	0.088%
8%	0.102%

THE DIFFERENCE BETWEEN NORMAL AND DIABETIC BLOOD

We have examined the blood of several normal and diabetic persons which, at one stage of the work, we thought showed a significant difference. When examined more closely, we think no such difference exists. The ratio of the amount of sugar found by using 12.5 per cent KOH in Fehling's solution to that determined by using 5 per cent KOH Fehling is striking.

Ratio in Normal Dogs

12.5% of KOH Fehling	5% KOH Fehling	Ratio
0.048%	0.140%	1:3
0.010%	0.067%	1:7
0.028%	0.070%	1:2.5
0.025%	0.092%	1:4
0.020%	0.110%	1:5
Average of 12		1:4

The influence of anesthesia, as is well known, raises the blood sugar, and if continued long enough, may cause a glycosuria. It also changes this ratio remarkably.

	12.5% KOH	5% KOH	Ratio
I. Normal	0.020%	0.110%	1:5
After 2 hours ether anesthesia	0.08 %	0.156%	1:2
II. After 10 minutes ether	0.052%	0.104%	1:2
III. After 3 hours ether	0.205%	0.260%	1:1.5

Ether always reduces this ratio, as does anything that raises the amount of sugar in the blood. In six dogs under ether for 1 hour, the ratio fell from 1:4 to 1:3. In a case of adrenaline glycosuria without ether, the ratio was practically 1:1. In one case of phlorizin diabetes, in which, as is well known, the blood sugar does not increase, the ratio did not change.

In normal human beings, the ratio is practically the same as in normal dogs.

No.	12.5% KOH	5% KOH	Ratio
1	0.025%	0.090%	1:3.6
2	0.035%	0.160%	1:4.8
3	0.082%	0.230%	1:3
4	0.082%	0.240%	1:3

Human diabetic blood again shows almost a ratio of 1:1 as the following results from 3 cases of diabetes will show.

No.	12.5% KOH	5% KOH	Ratio
1	0.172%	0.256%	1:1.5
2	0.290%	0.307%	1:1.05
3	0.670%	0.730%	1:1.1

Some of this diabetic blood was allowed to glycolyze in the incubator at 40° C. until the sugar had all disappeared, and then a known amount of glucose was added. Of the added glucose, in two cases only, 40 and 60 per cent were recovered, when analyzed by the strong alkali Fehling, so that if there be such a thing as an amboceptor, it acts, so far as we can make out, as strongly in diabetic blood as in normal.

Closer analysis leads us to reject the amboceptor theory and to incline towards the opinion that there is no difference in the state of the sugar in normal and diabetic blood. The difference in the amount of blood sugar found when we use a Fehling solution which contains 12.5% NaOH and the amount found when we use 5% NaOH Fehling is enormous. The difference between the results obtained by the stronger Fehling solution and the Lewis-Benedict is about the same as between the two concentrations of the Fehling solution. When all these methods are tested on a solution of dextrose in water, there is very little difference in the results obtained by all of these methods. Consequently, at first one is inclined to believe that the sugar in the blood exists in a different state than sugar in water. The results obtained from a blood filtrate suggest at first that some of the blood sugar is in a combined form. The substance to which the sugar may be combined might be considered as the postulated amboceptor. On analysis, however, it is seen that the difference in the results of these methods depends on an interfering body. This body apparently interferes more in strong alkali than in weaker, and it is just as active in the blood of pronounced diabetes as in the normal blood. It does not interfere with the Lewis-Benedict method.

In the worst case of diabetes cited, the blood sugar found by using 12.5%

NaOH Fehling solution was 0.670% while that found by using 5% NaOH was 0.730%. The ratio here is approximately 1:1. This might mean that no amboceptor was present. The actual difference in the amount of sugar in this case as determined by the two concentrations of Fehling's solution is 0.06% or exactly the amount of difference in normal blood. On account of the low initial figure of normal blood, the ratio would be about 1:4. Consequently we are forced to admit that this ratio does not mean that there is a difference between diabetic blood and normal blood.

It has been shown above that after glycolysis diabetic blood will act towards added sugar just as normal blood, and the reason the added sugar can not be recovered by the Bertrand method is because of an interfering body.

The nature of this body is organic because blood ash does not interfere. Alkali normally dissolves some cuprous oxide. In the presence of this organic substance which picric acid does not remove, the solvent action of the strong alkali is increased. Looked at in this way, there is nothing to indicate an amboceptor or sugar in the blood in a combined form.

Since direct examination shows that the sugar of the blood exists as a water solution, we have sought by indirect methods to gain further evidence either for or against this opinion. One method of attack was to determine whether or not tissues or cells can utilize free sugar.

Ludwig[41] thought that oxidation took place in the blood. We now know that the blood sugar is oxidized by the form elements of the muscles and glands mainly, and only in minute amount in the blood. No oxidation takes place in the serum other than that due to the alkalinity of the solution. I have kept serum for ten days in the incubator at 40° C., without an appreciable loss of sugar, while in the whole blood, the sugar under the same conditions disappears completely in twenty-four hours. When the blood is laked, glycolysis stops. The corpuscles contain very little, if any, sugar. All these facts are in keeping with the opinion that the blood sugar is in free condition. That the oxidation takes place in the form elements, and that the free sugar can be directly oxidized, is supported by many other facts. First, in fermentation of yeast, there is no action outside the cell, no extracellular enzyme. This may be easily shown by adding yeast to a solution of dextrose, and, when fermentation is progressing rapidly, by filtering the solution through an ordinary filter paper. Filtration by straining out the yeast cells stops fermentation instantly. Again, blood flowing through living tissue loses some of its sugar, as Bernard has shown. The amount is small, but may be increased by muscular contraction; in fact, this is a method sometimes used to free an animal of glycogen. By use of strychnine tetanus, strong muscular contractions, or other means leading to extreme fatigue, the liver becomes glycogen-free. That the free sugar can be used in this way is shown by perfusing isolated organs, as the heart, or muscles. Years ago, I perfused the isolated hind legs of animals, keeping one leg in each case for control, and while the perfusion was proceeding, kept the muscles working by electrical stimulation. The results clearly showed a loss of sugar, apparently by direct utilization. This work was done when we believed that the blood sugar existed in a colloidal state. By more refined methods, more exact figures may be obtained, but we think that the facts will remain essentially the same.[42]

The method used consisted in perfusing the organ under investigation with blood containing a known amount of sugar, and determining the loss of sugar during the perfusion. One hind leg of an animal was perfused and the other used as control. Account was taken of the amount of glycolysis that the blood would have undergone during the time of the perfusion, the changes in the glycogen content of the leg, the changes in the sugar content of the leg, and the loss of sugar from the blood. While the perfusion lasted, the leg was stimulated through the nerve with an electric current. The idea of this was to increase the amount of sugar oxidized.

During the perfusion there was never found any formation of glycogen. *Apparently the power of glycogen formation is lost before the power to oxidize sugars.* This point would indicate that glycogenesis may be most at fault in diabetes. The monosaccharides only were oxidized. When the leg failed to respond to electrical stimulation, the power to oxidize sugars was greatly reduced and even lost. Apparently the living protoplasm is the active agent. A few of the results are presented calculated for 100 gm. muscle:

Sugar used	Time of perfusion	Amount of sugar used
Dextrose	60 min.	15 mg.
Levulose	60 "	25 "
Levulose	60 "	33 "
Galactose	60 "	81 "

The heart while contracting also uses sugar. This has been shown by Knowlton and Starling,[43] by Rhode[44] and by Locke and Rosenheim.[45] Camis,[46] however, claims that it may not have been the perfused sugar that was directly oxidized, but the stored glycogen of the heart itself. There seems little basis for such contention. Our own work shows that the sugar may be used directly by the contracting muscle.

The sugars that are used directly are those that are directly fermentable by yeast. The products are the same in both cases, and I believe the mechanism is also the same. In each case the living cell is the active agent. This can be readily shown in the case of yeast by simply passing through ordinary filter paper a solution of sugar that is undergoing rapid fermentation. The filtrate at once ceases to evolve gas. It is true, as Buchner[47] has shown, that when the yeast cell is ground up the filtered juice will still cause fermentation, but the significance of this fact has been much overestimated, and the time that such fermentation will proceed is very short indeed, and suggests that it may be due to living particles of the cell protoplasm. Similar, but more marked, is the depressing influence of disintegration of animal tissues on the oxidative properties. This, I think, was the cause of Cohnheim's[48] failure to demonstrate the synergistic action of muscle and pancreas extracts on the oxidation of glucose. There can be no doubt about the interrelated action of the pancreas and muscle in the normal animal, but since it is the living cell that oxidizes glucose, or at least the enzyme in the living cell, we should not expect in all cases that the same action can be demonstrated when the cell is disintegrated. The conjecture was legitimate when isolated enzymes were considered as the sole source of oxidation. The method of Knowlton and Starling[49] on the contracting heart,

or of Macleod[50] on the eviscerated animal, seems a better method of attacking this problem.

Lavoisier demonstrated that the results of oxidation of foodstuffs in the body is the same as oxidation out of it. Sugar oxidized in a calorimeter or in the body, produces the same number of calories or heat energy, and inside the body this heat is available for the body. This does not mean that the mechanism of the oxidation is the same in the two cases. As a matter of fact, we do not know how such simple oxidations as the burning of carbohydrate or magnesium take place. We know that as the result of such oxidation, heat is liberated; but as to how it is transferred to the body, we are still in the dark.

This is one point in which the two types of oxidation may be the same. Moritz Traube[51] considers that the simplest oxidation is an hydrolysis, that is, oxidation will not take place in the absence of water. This may be represented by the following formulæ:

$$M + O + H_2O = M\begin{matrix}OH\\OH\end{matrix} = MO = H_2O$$

and the water can again be used in the process. The oxidations in the body are probably also hydrolytic, but must be more complicated in nature than the simple burning of a magnesium ribbon, or a splinter of wood in the air. It is generally accepted that foodstuffs burn, and are used by the body only after they have become an actual part of the body. In other words, it is the living matter of the body that burns. It is the disintegration of the food molecules after they have become a part of the biogen or living nucleus that renders the body such an economic machine. The rupture of the carbohydrate is an exothermal reaction, and the energy when liberated is already a part of the machine. Concerning what the living molecule or biogen is—if such exist—no satisfactory opinion has yet been advanced. The characters of mitochondria suggest that they may be of fundamental importance,[52] and approach the characters of the hypothecated biogen.

Since the greater part of the digested carbohydrate is changed to glycogen in the body, and this again to glucose before utilization by the tissues, it is of interest to know what conditions modify glycogenolysis. As is well known, some agents increase the blood sugar while others reduce it. I wish only to mention a few of these which we have recently investigated. In the first place, what is the effect on the blood sugar of modifying the circulation through the liver? Bernard[53] thought increased liver circulation was the cause of diabetes. E. L. Ross and I,[54] accordingly, studied the effects on the blood sugar of (1) ligation of the portal vein, (2) ligation of hepatic artery, (3) simultaneous ligation of both portal and hepatic, (4) hyperarterialization and, (5) Eck fistula and, (6) reversed Eck fistula.

The results are shown in the accompanying tables. Hyperarterialization of the liver was accomplished by first making an Eck fistula and then turning an artery into the portal vein or its branches. The results were obtained from acute experiments of one hour duration, and clearly show that ligation of the portal vein, or greatly increasing the arterial blood going through the liver, are the only operations to cause any marked change in the blood sugar, and these changes are never sufficient to cause glycosuria. Eck fistula and the reverse,

also in animals that were kept for months by Robert Keeton,[55] had little influence on the blood sugar. We hold, therefore, that no conceivable change in the circulation can be of much importance as a causation of diabetes, unless the change secondarily involves the pancreas or some other organ. It is well known that if circulatory changes involve the pancreas sufficiently to produce inflammation of that organ, glycosuria may result. Uncomplicated changes in the liver circulation can be of only small import as a direct factor. This is of interest, in view of the fact that Bernard thought piqure, or puncture of the fourth ventricle acted to cause glycosuria by changing the liver circulation. It is now known that unless the adrenals and liver are both intact, puncture alone will not cause glycosuria.

The average of the results of modifying the circulation are:

No. of Expts.	Percentage of Dextrose in Blood Before ligation	After ligation	Length of time ligated	Actual sugar increase
Portal ligation 8	.088%	.145%	59 min.	0.056%
Hepatic ligation 8	.105%	.124%	60 "	0.019%
Portal and hepatic 8	.126%	.114%	50 "	−0.012%
Hyperarterialization	.176%	.238%	50 "	0.062%
Hypervenosity 5	.141%	.157%	60 "	0.016%

Summary Table

	Increase calculated by difference in percentage before and after operation	Increase expressed in per cent of sugar content before operation
Ligated portal	0.056%	62%
Ligated hepatic	0.019%	18%
Portal and Hepatic	0.012%	− 9%
Hyperarterialization	0.062%	35%
Hypervenosity	0.016%	11%

CONDITIONS REDUCING THE BLOOD SUGAR

Hypoglycemia has not been considered so important as hyperglycemia. The reason is very apparent. Diabetes is so common and so fatal a disease that all phases of it have attracted attention. When, therefore, it was found that the blood sugar is usually increased and that when it reaches a certain concentration, it passes into the urine, the cause of the disease was thought to be closely associated with the blood. On the other hand, we know of no disease where hypoglycemia is considered so important as the hyperglycemia of diabetes. The methods which reduce the blood sugar are worthy of attention for the following reasons: First, they may illuminate the process of normal sugar metabolism; and, second, they may reveal something of value in treatment. While such possibilities exist, it must be confessed that all of the known methods of blood sugar reduction, with the exception of Allen's method[56] are of much more immediate injury to the animal than the most rapidly progressing hyperglycemia.

The conditions that may cause a reduction of the normal sugar concentra-

tion are: extreme exhaustion,[57] adrenal insufficiency,[58] Addison's disease,[59] thyroid insufficiency,[60] poisoning by phosphorus,[61] and hydrazine[62] or phlorizin,[63] high section of the cord,[64] anaphylactic shock,[65] intercurrent affections,[66] moribund states,[67] and the injection of foreign proteins.[68] The striking physiologic changes that result from the application of most of the methods are: low blood pressure, splanchnic dilation, depleted vitality, and, in larger doses, a moribund state. In none of them is there the remotest suggestion of anything that operates for the welfare of the organism. Various artificial hyperglycemias and glycosurias have been reported to be lessened by the administration of certain glandular extracts or salt solutions. Stenström[68] found that pituitrin lessened adrenalin hyperglycemia. Dresel[69] reports that extracts of the pituitary, thyroid, ovary, or pancreas, when injected with epinephrin reduce the height of the hyperglycemia. Miculicich[70] found that hirudin inhibits, and Glaessner and Pick[71] found that pancreatic juice and Witte's peptone lessen epinephrin glycosuria. Bock and Hoffman[72] showed that salt glycosuria is lessened by the further action of the injected saline. It is now known that calcium chloride will lessen salt glycosuria. Underhill[73] found that the glycosuria produced by morphine, pyridine, etc., is reduced by the free administration of oxygen. Other cases of the kind are reported by Allen.[74] It should be stated, however, that the whole subject is unsatisfactory and contradictory to such an extent that many substances are recorded by some to produce hyperglycemia or glycosuria, while according to others the same drug causes hypoglycemia. Phosphorus, salt solutions, and peptone are among such drugs. Also, in most moribund states the blood sugar increases immediately before death.

In making blood transfusions from one normal animal to another, we have noticed that there is a general tendency for the blood sugar of the recipient to fall. We have since found that this is a common result of the intravenous injection of proteins, and especially of peptones, although Henderson and Underhill[75] have reported glycosuria following the injection of peptone. We can not confirm their results; and look upon the glycosuria they describe, not as a peptone, but as either an ether glycosuria or as an exception to the regular peptone action. Similar exceptions may be seen in those cases in which peptone hastens, rather than retards the coagulation of the blood.[76] The whole subject of the action of sera, organ extracts, and toxins on the sugar content of the blood is in an unsatisfactory state, and the action perhaps varies with conditions.

In our work we used Witte's peptone chiefly but we obtained similar results with silk peptone, and with gelatin; and in several cases of anaphylactic shock, which resembles peptone action, we found a hypoglycemia.

For the study of peptone hypoglycemia we used dogs entirely. The number of animals used without anesthesia was thirty. The average weight was 10.6 kilos and ranged from 4 to 18.5 kilos. The average dose of peptone, which was 3.7 grams, was given intravenously in about 20 c.c. of water. The average normal blood sugar content was 0.65 per cent. In from two to five hours after the injection of peptone, the blood was again analyzed. On the average, the time was three and one-half hours. The blood sugar by this time on the average, was reduced to about one-half the original content, or 38 per cent. We found a great reduction also when an anesthetic was used. The cause of the reduction

is not clear. Perhaps it is due to changed circulation through the liver, although this can not be sustained by experiment. It may also be due to a paralysis of the normal mechanism of glycogenolysis. The liver still contains considerable quantities of glycogen. In three cases which we analyzed, the glycogen content of the fresh liver was 0.45 per cent, 1.91 per cent and 3.18 per cent. The blood sugar in the last case was lower than any of the others. It may be that there is a greater utilization of the sugar in the circulating blood and lessened glycogenolysis, but this we have not investigated. The most common changes after peptone injections are low blood pressure and the unsatisfactorily explained condition known as extreme fatigue, or shock. None of the measures that reduce the blood sugar by the use of drugs offers the slightest hope for the treatment of diabetes. When, however, we reflect that almost all the definite knowledge we have regarding diabetes is the product of less than one hundred years of research, there is much to be hoped from a continuation of the study.

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WAR DEAFNESS AND ITS PREVENTION—A REPORT OF FURTHER TESTS UPON PREVENTIVES*

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METHOD OF TESTING

THE tests here reported were made by means of the tambour method. Using the rubber ear previously described to hold the various preventive devices, connection was made from the "medial" end of the canal to a tambour and the records of the amount of the detonations that passed the preventives were made on smoked paper instead of as physiologic reactions in animals' ears. The guns used as sources of the detonation waves were clamped in a constant position, therefore the relative amount of force that passed the various devices is indicated by the height to which the tracer of the tambour moved. Blank cartridges were used; these vary considerably, due probably to the packing of the wadding. But the average of several shots gives fairly uniform results, so it has not been checked up with ball cartridges, which would probably be more uniform in the individual loads.

DETAILS OF ARRANGEMENT OF APPARATUS

The rubber ear was placed in the wall between two rooms which are connected by a third room. The wall is an ordinary lath and plaster wall, with an opening 12 by 24 inches in it. This opening was closed by a board one inch thick in which a hole two inches in diameter was drilled. The rubber ear was fastened into a piece of board one inch thick and six inches square by drilling a hole in the center of the piece and then sawing it across the hole and carefully trimming away parts until the ear fits properly into the opening. The halves were firmly fastened together by strips screwed on, and the whole piece, containing the ear, was fastened firmly onto the board partition with the ear in the two-inch opening. This opening was beveled so as to be about four inches in diameter at the surface of the board. It was feared that this wall would permit sufficient shock to pass to affect the tambour by acting on the upper surface of the membrane, and that it would be necessary to resort to another location after preliminary tests, but such action on the tambour was not observed.

The tambour that was found to be suitable for such records as were desired is a standard Lombard pattern with a very light rubber membrane 18 mm. in diameter and a very light bamboo lever with a paper tracing point, the whole lever arm being 9 cm. long. It is pivoted about 2.5 mm. from the axis. The force acts during a very short time and doubtless the momentum developed in the tracing lever serves to carry it beyond the point where the force ceases to

*Submitted to the National Research Council. From the Department of Anatomy, University of Michigan Medical School, Ann Arbor, Mich.

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act, thus drawing out the membrane of the tambour. Instead of being the drawback which this would be with many kinds of tambour work, it serves here to indicate the very factor desired; namely, the force of the detonation wave that strikes the membrane of the tambour (in actual use of the preventives it would be the tympanic membrane). As expected, the writing lever rebounded a few times after the first movement, tracing a curve with very sharp apices and entirely without secondary components. It is only the distance of the first apex above the level of the base line that is of value here, and this is the distance referred to in the tabulated results. This, of course, indicates the compression phase of the wave;. the method is not suited to obtaining the rarefaction component also, because of the momentum of the system.

Detonation waves of three degrees of force were used; they were generated by the firing of revolvers of 22, 38 and 44 calibers. The cartridges were blanks in all cases and were Peters' 22 shorts, U.M.C. 38 long Colt's, and U.M.C. 44 Colt's. The guns were fastened in a constant position by means of clamps attached to a plank fastened to the floor in an upright position. The detailed position varied slightly for the different guns, but was constant for the series of experiments with each gun. The 22 caliber revolver was fastened with the muzzle at a distance of 6 cm. from the orifice of the meatus of the rubber ear, with the gun pointing downward 25 degrees and toward the plane of the wall in which the ear was fastened at an angle of 12 degrees from parallel. The distance from the muzzle to the point where the line of fire intersects the line projected perpendicular to the wall at the meatus is 5 cm. It will be seen from the above that the shooting was as close as could be done without actually entering the ear with the flash of burning powder. The detail measurements for the positions of the larger guns were slightly different, but the essential arrangement was the same; all pointed slightly toward the plane of the wall with the ear at from 6 to 7 cm. from the muzzle and just out of line of the flash of powder.

As was stated in the animal experiments report, I fully realize that the detonations used are not of the initial intensity of those caused by the firing of artillery or by the explosion of shells or mines, but the distances used compensate to such a degree that with the animals severe injuries resulted when they were not protected; and in these physical tests the detonation force is constant except for cartridge variation; this permits a relative testing of the devices even though the absolute force of the detonations is less than that caused by larger explosions. Both methods of testing are well suited to using near large guns, and such tests could be readily performed at target practices or munitions proving grounds if proper authority and a few laboratory facilities were provided. Such tests would be an interesting check on these.

The guns were fired from the tambour room by means of a cord acting over pulleys, so that I was able to be with the recording apparatus and be sure that it was operating properly at all times. The kymograph speed used was such as to spread the apices of the after-vibrations sufficiently to avoid any confusion with the primary movement.

PREVENTIVES TESTED

The names of the preventives tested are listed in Table I, descriptions of the eight which were also used in the animal experiments have been given in that report.* Besides these eight, cotton soaked with water and the Elliott "Swimmer" were tested. This latter is a device manufactured by the same firm that makes the Elliott Perfect Ear Protector and is sold by them for use by swimmers to keep water out of the ear and to prevent unpleasant sensations when diving. It is very similar to the ear protector and consists of a celluloid core with three spaced discs of rubber; two light ones enter the meatus, and the third heavy one rests on the orifice. The celluloid center does not have a hole in it as does that of the protector.

RESULTS

Three or four shots were fired with each device in each series except that with the 44, where only two shots were fired in some cases. The averages of the heights of the first apices of the resulting curves are given in Table I. In Table II are presented in graphic form the results with the 44 caliber revolver, with which all the preventives permitted enough of the force of the waves to pass to give measurable tracings. All measurements were made with a Bunne curve analyzer with a vernier permitting readings accurate to 0.05 mm. Because of the simple nature of the curves themselves, it is not necessary to figure them in this report, since it is only the height of the first apex that has value in these tests.

TABLE I

AVERAGE HEIGHT IN MILLIMETERS OF THE CURVES RESULTING FROM THE SEVERAL SHOTS WITH EACH DEVICE*

PREVENTIVE DEVICE IN MEATUS OF RUBBER EAR	22 CALIBER REVOLVER	38 CALIBER REVOLVER	44 CALIBER REVOLVER
Nothing (ear open)	25.76	50.77	61.67
Dry cotton (packed firmly)	18.58	33.27	56.50
Elliott Perfect Ear Protector	17.76	31.22	54.65
Elliott "Swimmer"	12.40	29.15	48.77
Wilson-Michelson instrument	24.05	14.86	13.42
Water-soaked cotton	....	3.84	1.17
Wax Cone of Italian Navy type	0.41	5.02	0.50
Mallock-Armstrong Ear Defender	0.18	0.27	1.02
Glycerin-soaked cotton	Trace	0.38	0.35
Vaseline-soaked cotton	Trace	0.07	0.30
Scientific Ear Drum Protector "Tommy"	0.13	0.27	0.27

*For the ammunition used and the arrangement of the guns and apparatus see the text matter.

DISCUSSION OF THE RESULTS

In considering these results it should be kept in mind that probably the movements of the tambour are not a direct proportionate measure of the force; but that of two movements, the greater indicates relatively greater force than that implied by the ratio of the movements because of the increasing tension of the membrane of the tambour. No attempt has been made to calibrate the forces needed to cause the movements given, because the suddenness with which

*Jour. Lab. and Clin. Med., Jan., 1918, iii, p. 226.

the detonation wave acts doubtless affects the movements as compared to that produced by a constant air pressure such as is used in calibrating. For the above reasons the results are fully recognized by me as indicating only the relative order of efficiency; not as being absolute.

The first thing noticed in studying the results is the division of the preventives into two distinct groups on the basis of the efficiency in stopping the force of the detonation waves. The ones that permitted the detonations to cause large movements of the tambour are the same ones that permitted the greatest injuries to the middle ears of animals. To these latter is added here the Elliott "Swimmer," which was not tested on animals. It is better than

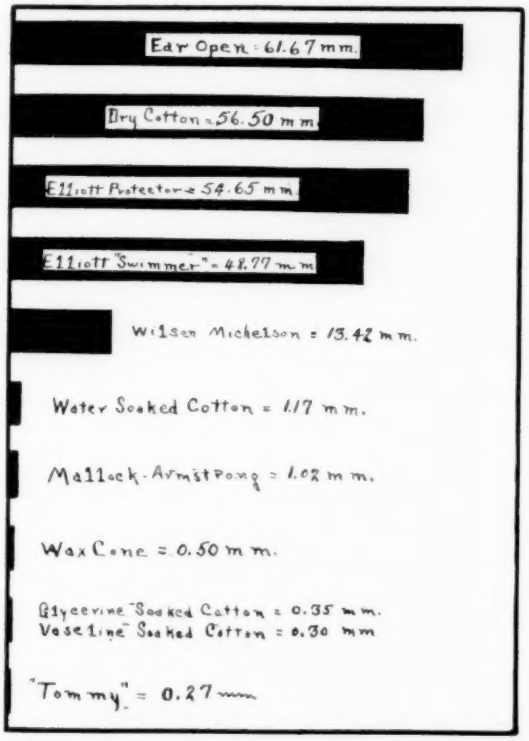
Ear Open = 61.67 mm.
Dry Cotton = 56.50 m m.
Elliott Protector = 54.65 m m.
Elliott "Swimmer" = 48.97 m m.
Wilson Michelson = 13.42 m m.
Water Soaked Cotton = 1.17 m m.
Mallock-Armstrong = 1.02 m m.
Wax Cone = 0.50 m m.
Glycerine Soaked Cotton = 0.35 m m.
Vaseline Soaked Cotton = 0.30 mm
"Tommy" = 0.27 mm

Chart II.—A graphic presentation of the results with the 44 caliber revolver.

the Elliott protector, but would certainly permit positive damage to the ears under conditions similar to those used with the animals.

On the other hand, the Scientific Ear Drum Protector "Tommy," which the middle ear conditions indicated as the best of the devices tested, has continued to be the device of choice. The Mallock-Armstrong Ear Defender is not quite so effective with these tests, but continues to be in the same group. To the objections for military use which I have previously urged against this device I have another to add now. The diaphragm of one of the pair of devices which I have has split and accordingly the device is greatly decreased in efficiency. With the damaged device in the meatus of the rubber ear, three shots from the 22 revolver gave tracings averaging 13.97 mm. While such splits

might not occur very often, yet when one did occur, the chances are entirely against the user knowing of it until he is injured because the diaphragm is partially concealed by the wire gauze. The metal diaphragm of the original 1915 patent is to be preferred so far as this score is concerned.

The glycerin- and vaseline-soaked cotton plugs have given better results here than in the animal tests; they are the only preventives that have not remained in the same relative positions in the order of efficiency as tested by the two methods. When opportunity arises, I hope to repeat the animal experiments with these soaked cotton plugs and see whether they duplicate the former results. As I remarked in the animal report, the universal availability of vaseline and cotton renders the degree of protection afforded by the mixture of importance to those to whom good mechanical devices are not available. These soaked cotton plugs cut down the hearing of ordinary sounds more than any of the other devices; this is, of course, an important objection to their military use.

The discrepancy in the results with the wax cones is probably due to the temperature. When the 44 was used, the room was very cool, while when the other series were run, the room was much warmer. I believe this is the correct explanation of the discrepancy. If so, it indicates a firmer wax for use in the human meatus, where a temperature warmer than that in the room would be obtained. Gianturco recognized temperature effects and suggested different mixtures for summer and winter use.

The discrepancy in the water-soaked cotton may be due to two factors; either a difference in the thoroughness with which the cotton was wet on the two occasions, which would be a factor impossible to control in actual use, or a more rapid evaporation may have occurred on the warmer evening. This latter is indicated by the fact that with the 38 the four shots fired gave successively increasing movements of the tracer, the actual measurements being 1.4, 3.35, 5.10, and 5.50 mm. Water-soaked cotton in the human meatus would so soon become dry cotton that it can not be recommended even though it is a fair protection when thoroughly wet. However, in an emergency it would certainly be advisable to wet cotton instead of using it dry and it would be well to rewet it frequently if opportunity could be found.

The behavior of the Wilson-Michelson device is interesting for it is both relatively and absolutely more effective with the heavier waves. It seems paradoxical at first, but when the structure of the device is considered, it is seen to be the logical result. The inertia and friction of the valve are more quickly overcome by the heavier waves and it is carried shut enough sooner to render the total energy that passes the valve less in the case of the more intense waves than with the slower action with the smaller waves. However, in all cases it acts too slowly to protect well; it will be recalled that with the animals it uniformly permitted severe injury to the middle ear parts with a 45 caliber pistol firing ball cartridges, with which the detonation was probably stronger than with the 44 blanks.

I would call attention to the fact that the animal experiments are the ones which indicate the devices that permit an injurious amount of detonations to pass; these physical experiments are of value in helping to place the preventives in their relative order of efficiency. The cochleæ of the animals, as ex-

plained in the middle ear report, will furnish a further check on the devices.

The fact that both methods of testing give concordant results indicating a relatively poor protection by dry cotton, by the Elliott Perfect Ear Protector, and by the Wilson-Michelson instrument in comparison with the protection afforded by other preventives leads me to express the wish that the proper military authorities will recognize the facts and take action to stop the use of the poor protectors by the troops and will furnish them with the better ones.

ANNOUNCEMENT

The method of testing here reported will enable me to make a determination in a few days after receiving it of the efficiency of any new device that may be submitted. Such a testing would place the new device in its relative order among the others and thus give a very fair indication of its effectiveness in protecting the ear itself. The more time-consuming animal testing could then be used as a check on the physical tests.

ACKNOWLEDGMENTS

I wish to here acknowledge my great indebtedness and to express my thanks to Dr. W. P. Lombard for the way in which he has placed the facilities of the physiologic laboratory at my disposal for these tests, and for the many helpful suggestions he has made in the course of the work. My thanks are also extended to Dr. Cope, Instructor in Physiology, for suggestions and aid with apparatus, and to Mr. P. M. Ireland and Mr. K. C. Kerwell, medical students, and to Sheriff Lindenschmidt for the use of revolvers.

A CONTRIBUTION TO THE PHYSIOLOGY AND PHARMACOLOGY OF CHELONIAN LUNGS*

By D. E. Jackson, Ph.D., M.D., and Mort D. Pelz, St. Louis, Mo.

THE peculiar anatomic development of chelonians render these forms especially well suited for the study of certain phenomena relating to the innervation, physiology, and pharmacology of the lungs. In the year 1863 Mitchell and Morehouse[1] published a paper describing the musculature and mechanical actions by which the respiratory movements are carried out in these animals. In the present investigations we have confined ourselves to a study of the nature and course of the nerves controlling the intrinsic muscles of the lung substance, and to a few preliminary observations on the character of the responses of the lungs to certain drugs. Ordinary fresh-water American turtles have been used.

The method employed in these experiments is new and was devised in this laboratory about two years ago.[2] In the year 1909 Prevost and Saloz,[3] working in Geneva, studied certain features of the nervous control and drug reactions of the lungs in the tortoise. The method used by them consisted in making a round trephine opening through the carapace over the anterolateral region and then placing tightly in the trephine opening a cork through which a glass tube connected with a recording tambour was passed. The lungs were then rhythmically inflated by an artificial respiration machine, the air being conducted into the lungs by a cannula tied in the bronchus corresponding to the lung under observation. In this manner a number of observations were made by these authors. Most of these we have corroborated, but in a few instances we have failed to obtain such results as were reported by them. In addition to their work, which, so far as the innervation was concerned, covered only the vagal constrictor fibers, we have found direct evidence of the existence of a definite set of sympathetic dilator fibers, and we have also obtained results indicating that certain constrictor fibers are to be found running in the sympathetic nerves.

The simplicity of this work, together with the small amount of apparatus needed and the definiteness of the results, render these experiments peculiarly well suited for ordinary class exercises. There are a few striking differences in certain drug reactions between mammalian lungs and those of the turtle, but from a teaching standpoint these may be very valuable by way of comparison.

Reference to Fig. 1 will show the chief features of the method used by us. The turtle is thoroughly pithed, both brain and cord. The plastron and viscera (except the heart and lungs) are then removed and the skeletal musculature is dissected out of the carapace as thoroughly as possible, care being taken not to puncture the lungs which in the meantime may well be partially inflated by means of a cannula tied in the exposed trachea. The vagosympathetic nerve trunks are isolated in the neck and the bronchus to the lung *which is not to be studied* is closed off with a bulldog clamp. The other lung is connected by tub-

*From the Department of Pharmacology of Washington University Medical School, St. Louis, Mo.

A preliminary report of this work was given before the Federation of American Societies for Experimental Biology at Minneapolis on Dec. 27, 1917.

ing to a delicate tambour having a bowl about one and one-half to two inches in diameter. A high magnification is used and the lung is blown up to about one-half its capacity. This puts the air tension in the lung and in the tambour on a balance and a contraction or relaxation in the lung will show an immediate rise or fall of the writing point of the tambour.

Fig. 2 shows a diagram of the lung innervation in the majority of turtles studied by us, but there are considerable variations in different specimens of the same species, while in a few varieties (especially snapping turtles) we have not been able to recognize the sympathetic dilator fibers. It is extremely probable,

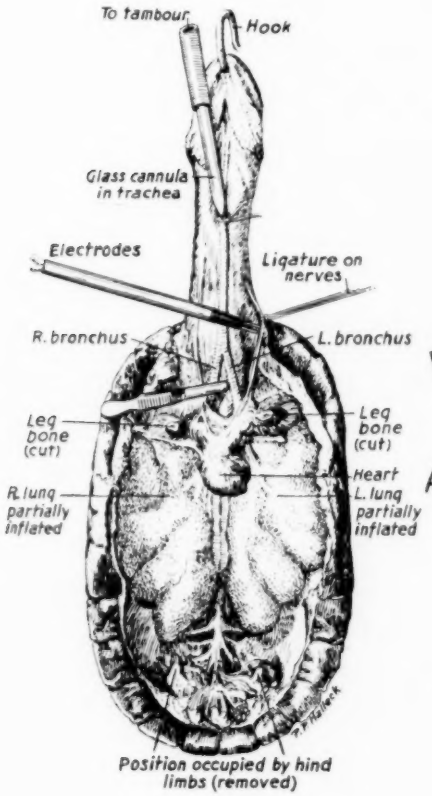

Fig. 1.

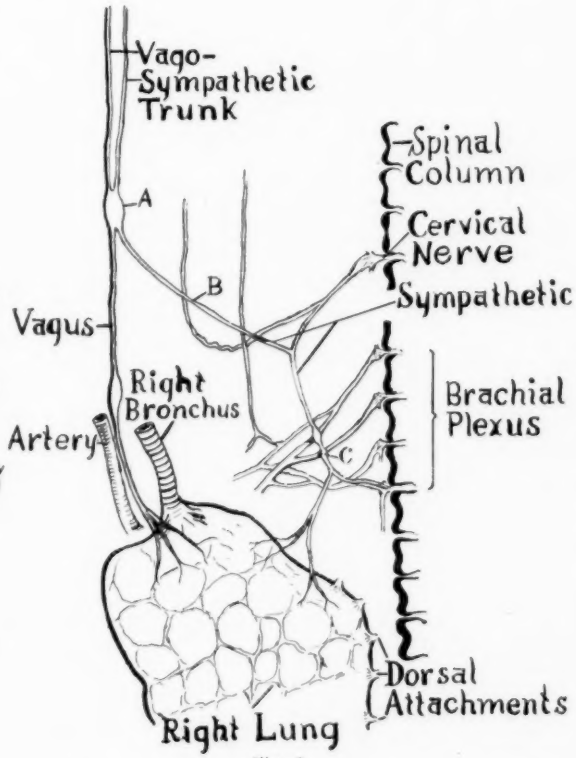

Fig. 2.

Fig. 1.—Dissection showing method of removing the plastron, viscera (except the heart and lungs and large blood vessels), and skeletal muscles. In this way each lung may be observed directly while the records are being made.

Fig. 2.—Diagrammatic representation of the innervation of the lungs in the turtle. Right lung, ventral view. For discussion see text.

however, that a more careful search would reveal the presence of these fibers in this species the same as in others. Apparently seasonal variations are also present in the action of these nerves. Probably these reasons are sufficient to explain our failure in some experiments to satisfactorily demonstrate either constriction or dilation of the lungs following electrical stimulation of the nerves.

At the ganglion marked *A* in Fig. 2 a very small sympathetic nerve bundle labeled *B* joins the vagosympathetic trunk. If the nerve *B* be stimulated with a *very weak tetanizing current*, a dilatation of the corresponding lung usually

follows, but if a strong induced current is used a contraction of the lung is much more frequently produced, and this contraction sometimes follows more vigorously and promptly than even that which is produced by stimulation of the vagus trunk itself. These results we believe justify the conclusion that in some turtles (if not in all) the sympathetic nerves carry both dilator and constrictor fibers for the lungs. These fibers are very small and difficult to isolate without injury,

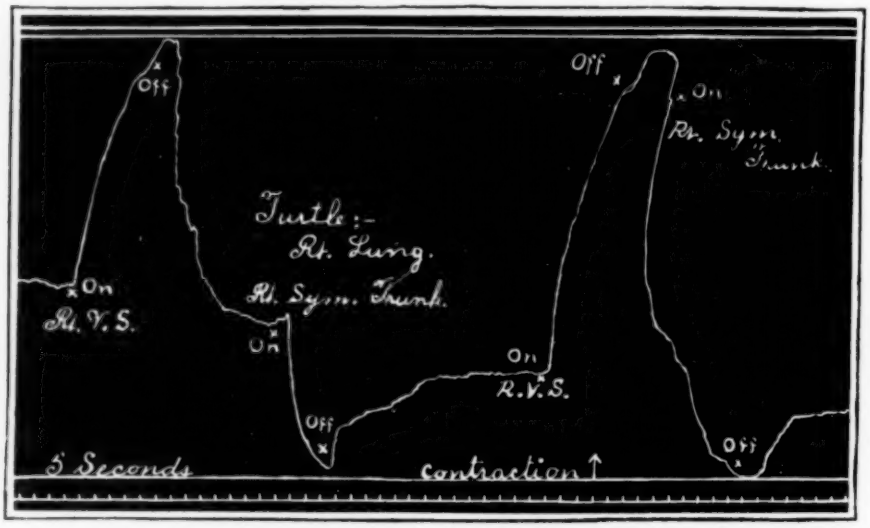

Fig. 3.—Tracing showing the contraction of the right lung following vagus stimulation, and relaxation produced by stimulation of the right sympathetic chain (at *C* in Fig. 2).

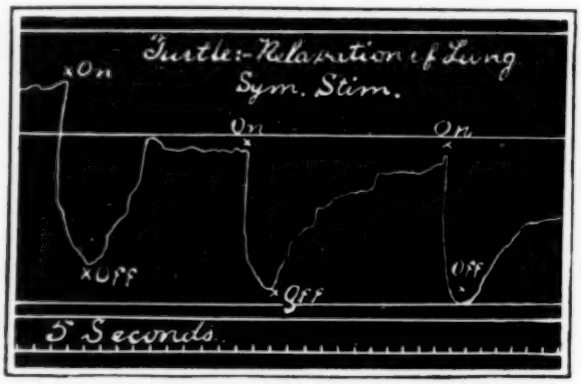

Fig. 4.—Tracing showing dilatation of the lung produced by sympathetic stimulation.

and this sometimes makes the experiment difficult or ends in failure. The dissection should be made by lifting the vagus nerve upward and outward from the base of the neck while the sympathetic branch marked *B* is followed down toward the main sympathetic chain at *C*.

If the main sympathetic chain in the region of the ganglion marked *C*, or on the ganglion just above this, be stimulated with a *weak tetanizing current*, a dilatation of the corresponding lung will be produced immediately. The dilata-

tion is most marked in the upper portion, that is, in the region around the entrance of the bronchus and in that part of the lung which lies lateral to this. Probably this is due to the better developed musculature in this region, for the posterior or caudal part of the lungs is much thinner and more membranous in its structure.

Figs. 3 and 4 show typical results obtained by stimulation of the vagus and of the sympathetic fibers. Stimulation of the vagus trunk causes stopping of the heart and lung contraction, but sympathetic stimulation, preferably always with a weak current, causes pulmonary relaxation and either no effect on the heart, or possibly occasionally some acceleration of the beat.

Regarding the response of the turtle lung to drugs, it may be said that nicotine, pituitrin, lobelin and veratrine cause marked contraction, usually of a prolonged nature. These drugs we have generally applied by injection with a small hypodermic syringe directly into the beating ventricle. The solutions are soon carried around to the lungs. None of the above drugs produce either a marked or prolonged contraction of the bronchioles in the dog. Codeine, pilocarpine, etc., act in the turtle apparently in practically the same manner as in the mammal.

We have repeatedly tried to get satisfactory results by the use of atropine and adrenalin. After atropine we have sometimes seen what undoubtedly appeared to be a direct muscular contraction, but we have not been able to follow this result up closely and it may possibly have been in each case the result of some technical error, but we hardly believe this to be the true explanation. With adrenalin we have not so far obtained a typical dilation of the lung as would occur in the case of the mammalian bronchioles. Possibly a constant perfusion of the lungs with a suitable solution to which adrenalin was added at the proper time might show a pulmonary dilatation, but the results are open to doubt at present.

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BACTERIOLOGIC FINDINGS IN OZENA—SECOND REPORT*

By Herbert C. Ward and Donald C. Beaver, Detroit, Mich.

THE etiology of chronic catarrhal infection demands more serious attention by the bacteriologist than it has yet been given. Available records repeatedly indicate that invasion of the upper respiratory tract may take place very early in life, children of three and four years becoming subject to pronounced forms of rhinitis from which they never recover. Our clinics register daily the ozenic and atrophic, the tuberculous or syphilitic adult of forty years. In fact there is no lack of fresh material or willing patients for the needs of serious investigation. Both are abundant from various sources and climates and of all ages.

In tabulating the cases coming to this laboratory for observation we have found it convenient to make the following distinctions. The diagnosis of atrophic rhinitis is indicative of atrophy, chronicity of discharge, crustal formation, and impairment of free nasal respiration. The term ozena refers to the obvious symptom of malodor. Inasmuch as ozena is closely associated with atrophic rhinitis and frequently presents all the symptoms of the same, it is here considered as a frequent symptom of atrophic rhinitis, and not ozena, a clinical entity. By definition, therefore, causative factors of the atrophic and also of the ozenic stage may not be identical. Investigation of this constitutes the objective of our study on some seventy cases. One hundred and fifty bacteriologic analyses were conducted as completely as the time and facilities were convertible for this work. The findings agree with those of the first paper† and have yielded very suggestive records.

The most comprehensive report published in this country is that of Horn of San Francisco. He suggests that ozena is not a clinical entity, meaning that more than one biologic agent may bring this condition about. After reviewing previous work and supplementing the same with a series of experimental vac-

Table I

B. mucosus capsulatus (Abel's bacillus)	appeared	in	42	cases,	or 84%
B. diphtheroids	"	"	37	"	" 74%
M. staphylococci	"	"	37	"	" 74%
B. Perez Type II (atypical)	"	"	34	"	" 68%
M. streptococci	"	"	31	"	" 62%
B. proteus	"	"	14	"	" 28%
B. Perez Type I (typical)	"	"	11	"	" 22%
B. influenza	"	"	9	"	" 18%
M. pneumococci	"	"	8	"	" 16%
M. catarrhalis	"	"	6	"	" 12%
B. coli and pyocyaneus	"	(each)	3	"	" 6%

cines, he proceeds to show that bacteriologically all his cases fall into two groups, one designated as the "Friedlander" and the second as the "Perez" group. Lit-

*From the Research Laboratory of Parke, Davis & Company, Detroit, Mich.
†Bacteriologic Findings in Ozena, First Report, Jour. Infect. Dis., Aug., 1916, xix, 153.

erature of the subject reveals similar conclusions from the earliest of the investigations and a previous report from this laboratory records identical findings.

Bacteriologic studies have aimed to include the most important groups of organisms so far as routine analyses and methods would permit. Table I includes the findings obtained from fifty cases diagnosed as ozena (atrophic rhinitis fetid). The groups are arranged in the order of their predominance in the fifty cases.

These cases are representative of conditions found in this country, having been taken from a wide radius as follows: Michigan, 25 cases; Louisiana, 5; Illinois, 2; Minnesota, 3; Ohio, 7; California, 4; and Kentucky, 4. Inasmuch as the material had to be shipped a long distance before being subjected to bacteriologic examination, the thoroughness of the analyses was limited to but one trial in the majority of the cases.

Reviewing Table I, it is evident that the preponderance of B. mucosus capsulatus is most significant. An organism similar to this and known as the Morax-Axenfeld bacillus appeared only a few times and is not, therefore, considered

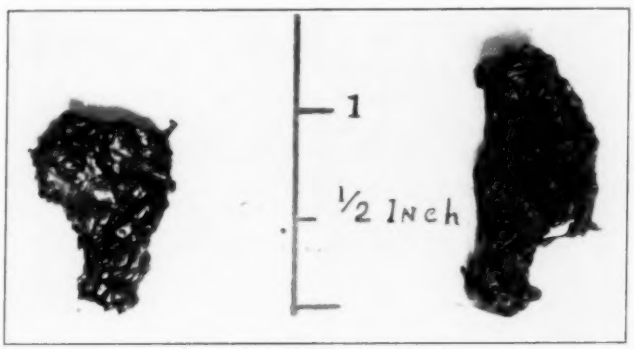

Fig. 1.—Fresh nasal casts formed in 48 hours from two girls, 10 and 12 years old, respectively, diagnosed as atrophic rhinitis cases. The records give histories of two and five years' duration.

as being important. It is easily confused with the B. mucosus capsulatus microscopically, but never culturally.

Diphtheroid bacilli have taken second place in the table. In two instances toxin-producing bacilli were abundantly present and the cases were classed as diphtheria carriers by the local board of health. The identification of such organisms from patients suffering from chronic catarrh is most suggestive for practical investigation bearing on the problem of diphtheria carriers. No work of this character has as yet been called to our attention.

The much discussed Perez group of bacilli has appeared in a higher percentage of cases than in the first census. The strains isolated do not conform, on careful study, to the reported type. Two groups are distinguished. Type I represents a class conforming to the standard strain. Type II represents atypical Perez strains, differing from the stock in their agglutination, fermentation, and motility tests. Neglecting the question of strain identity for the moment, the significant fact appears that some representative of the Perez group is found in forty-five cases out of the fifty. Of additional value is the discovery that those cases contain no Perez types, but in their place, bacilli set down as B.

proteus vulgaris. The Perez and the proteus groups are, therefore, responsible for the malodor of ozena cases.

The remaining groups of organisms appear at present to be of little or no significance.

Supplementary to the fifty ozena cases just reviewed, the following group,

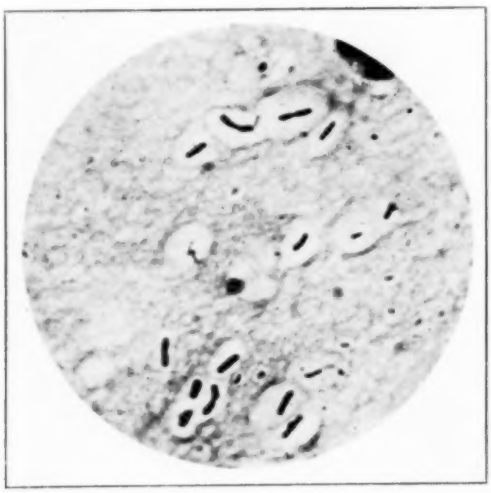

Fig. 2.—Original nasal mucus field. B. mucosus capsulatus types as seen with the Gram stain.

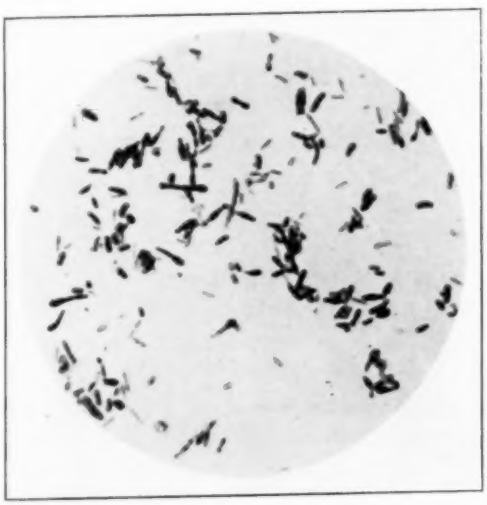

Fig. 3.—Original fields filled with the omnipresent representatives of the B. diphtheroids.

diagnosed as "atrophic rhinitis," and differing from the former only by the absence of odor, is tabulated. Material for bacteriologic study was received from: Michigan, 14 cases; Kansas, 1; Minnesota, 2; Illinois, 1; Ohio, 1; Indiana, 1; and Wisconsin, 1, making a total of 21.

TABLE II

B. mucosus capsulatus	appeared	in	17	cases,	or	81%
M. staphylococcus	"	"	16	"	"	76%
M. streptococcus	"	"	14	"	"	70%
B. diphtheroids	"	"	12	"	"	60%
B. influenza	"	"	3	"	"	15%
M. catarrhalis	"	"	3	"	"	15%
B. spore bearers	"	"	2	"	"	10%
B. pyocyaneus	"	"	2	"	"	10%
B. Morax-Axenfeld	"	"	1	"	"	5%
B. Perez	"	"	1	"	"	5%
B. proteus	"	"	1	"	"	5%

Two facts are strikingly evident; first, that the capsulatus group is the most abundant; and, second, that the Perez and the proteus representatives are the least abundant. In this connection it is to be noted that the patients when examined at the clinics showed no odor. The nasal discharges, when incubated, developed no odor (except in two cases) and yielded no cultures giving odors. The results suggest that the existence of a Perez-proteus infection can be recognized in the majority of cases by clinical examination alone, and in such instances bacteriologic examination is unnecessary.

A summary of our study shows that the group of organisms known as the B. mucosus capsulatus (Friedlander's bacillus, Abel's bacillus) is preponderant and may be pathognomonic in cases of chronic rhinitis. This group is also independent of the action of putrefactive bacteria. In cases in which the symptom of malodor exists, a different class of bacilli known as the Perez group is most abundant and all cases show either one or both of the Perez and proteus groups present as causative factors of this condition.

Cases of chronic catarrh may harbor pathogenic organisms of other species such as the diphtheria bacilli and contribute to the distribution of similar infections.

A CASE OF SYMMETRICAL PERIPHERAL GANGRENE*

By Charles E. Kiely, M.D., Cincinnati, Ohio.

PATIENT No. B. 4534, age 39 years admitted to the First Neurologic Service of the Cincinnati General Hospital on June 19, 1917. His chief complaint was "nervousness" and extreme tenderness with marked cyanosis of the hands. He was under the effect of alcohol.

The history developed the following facts; he had had the usual infections of childhood; at fourteen contracted a hard chancre which was treated by internal medication only and followed by an inconspicuous secondary macular eruption on the legs; admitted gonorrheal infection twice; had pleuropneumonia at 37; had been drinking to excess for five or six years.

The history of the present complaint was that for five or six weeks prior to admission he had been more than ordinarily excessive, averaging about one quart of whiskey daily. About two weeks before admission he had taken five migraine tablets for headache in about forty-eight hours. Two days before admission both hands began to turn blue and became equisitely tender to touch. He was an electrical engineer by profession and could recall using no reagents which might have produced a local condition.

Physical examination revealed a fairly nourished and developed conscious adult white male whose most conspicuous objective symptom was a marked cyanosis of the more distal portion of the hands and of the fingers, as well as of the corresponding portions of the feet and toes. The lips were only slightly ashy, and the rest of the body surface of a grayish tinge.

In the cyanotic areas the hands and feet were very cold, and at this first examination generally more tender to light than heavy pressure, though even then deep pressure on the thenar muscles was extremely painful. Subjective pain was constant and severe, and he could only find relief by constant application of ice bags and in addition morphine became a constant necessity soon after admission. At all times both pulses could be felt at the wrist and ankle, nor did the vessels on palpation give the impression of sclerosis. Blood pressure was 134 systolic and 100 diastolic. Further notes on the physical condition are: purulent nasal discharge; tongue heavily coated; teeth and gums neglected with marked pyorrhea and very offensive breath; pharynx congested; chest symmetrical but narrow with whistling rales heard over both lungs posteriorly; heart action regular, not enlarged, sounds easily audible and without murmurs; abdomen negative.

Mental Examination.—Patient was conscious and cooperated well. His attention was good and orientation for time, place, and personality correct. Emotional attitude was in proportion to his situation. Cerebration commensurate with his station and opportunities.

Neurologic Examination.—Smell, taste, sight and hearing normal. The pupils reacted rather slowly to light, by both direct and indirect illumination,

*From the Pathological Institute of the Cincinnati General Hospital.

but well on accommodation. There was no squint, ptosis or nystagmus, ocular movements were free in all directions.

The jaw did not deviate from the median line on opening the mouth. Both masseters contracted equally and with normal power on voluntary effort. The corneal reflex and facial sensation were undisturbed. All facial folds were normal, and the periorbital and perioral muscles contracted normally in voluntary and emotional response. In articulating there was some slight indefinable thickness of speech, but deglutition was normal. There was nothing to suggest abnormality of the muscle supplied by the eleventh nerve, and the pulse rate was not subnormal. The tongue protruded in the median line on voluntary effort, showed some tremor but no atrophy. The somatic muscles were of normal tone and development, and movements of the limbs were of proper range, power, and coordination.

Sensory System.—Heat, cold, pain, touch, and position sense were normal, with the exception noted above for the thenar muscles. The skin reflexes were absent. The tendon reflexes showed a slight symmetrical exaggeration, there was no clonus, Babinski or Chaddock.

The urine was cloudy, red, with specific gravity of 1,012, neutral and gave no sugar reaction, but a marked albumin ring. Microscopically it contained blood and hyaline casts. Red count, 3,460,000; white count, 7,600, hemoglobin, 87 per cent (Sahli): A spectroscopic examination of the blood showed hemoglobin bands, but none of methemoglobin.

The patient was seen on the twenty-fourth of June by Prof. R. S. Morris, who gave the following opinion:

"No petechiæ. Heart not enlarged. Sounds strong and clear. Lungs clear on percussion. Vesicular breathing throughout. An occasional squeaking rale over both lungs. In upper left interscapular space, a few crepitant rales. Radial pulse regular, good volume, vessel walls feel about normal.

"*Abdomen.*—No localized rigidity, no masses. Tympany in flanks. Spleen not enlarged by palpation or percussion.

"Gangrene of hands and beginning gangrene of feet suggest a localized endarteritis. An arterial thrombus would seem to be a possibility, especially in view of the blood in urine and stools, but the absence of petechiæ, and the symmetrical involvement are against it."

In the next two days the patient's general condition became worse and the duskiness of the skin became more general and the fingers as far down as the middle phalanges developed a typical gangrene of the dry type. A line of demarcation forming across the middle of the hand. The respiration became of the Cheyne-Stokes variety and on June 26 the patient died.

Clinical Diagnosis.—Acute and chronic alcoholism; spontaneous symmetrical gangrene.

AUTOPSY (J. S.)

The body was that of a well-developed, well-nourished adult white male about 40 years of age. Postmortem rigidity was present and well marked. Postmortem lividity was brilliant over the back and buttocks. The scalp and skin over the face and neck, ears and scrotum were markedly cyanotic. The

lips and gums were also cyanotic. There were numerous other large areas of cynanosis over the arms and legs. The toes were not cyanotic, but the great toes showed areas of hyperemia which were of a dark red color and tended toward a blue color along the edges. There was no evidence of any shriveling or hardening of any of the toes.

The fingers and thumbs of the hands were markedly cyanotic, shriveled, rigid, and flexed. The nail-beds were very cyanotic. The ends of the fingers were dark blue (almost black), hard and dry as far back as the proximal end of the middle phalanges. Here there was a distinct line of demarcation beyond which the color was of a lighter blue and the skin was softer and the joints were not so rigid. This area extended backward over the fingers and hands to about a half inch from the proximal ends of the metacarpal bones. This area extended farther upon the sides of the hand, as far up as the lower end of the ulna on the ulnar side and as far up as the lower end of the radius on the radial side. Here there was a second distinct line of demarcation beyond which the skin was somewhat cyanotic but not as deeply so as distally to this line. The flexed fingers were so rigid that they could not be straightened out. The blood vessels, especially the veins, in these cyanotic areas contained blood clots which had completely filled the vessels.

The cranium and spinal canal were opened and the brain and cord were removed. There was some edema of the brain and a moderate degree of congestion of the vessels of the brain. No lesion in the cord or brain could be noticed by the external examination, and gross section (C.E.K.) showed no abnormalities.

Upon opening the chest, the lungs partially collapsed. The left lung was free from adhesions and crepitated in its lower lobe, but had a shotty feel throughout its upper lobe. Upon section, the cut surface presented an edematous and slightly congested appearance and many miliary tuberculous nodules were visible in all stages in the upper lobe. The right lung was bound down at its apex and to the diaphragm by old fibrous adhesions. The cavities were easily seen in the apex. The largest of these cavities measured about 2 cm. in diameter. This lung was also crepitant in its middle lobe and felt shotty in the other lobes. Upon section, many tuberculous nodules were visible and cavities had been found in the upper lobe only. These cavities had thin walls. The middle lobe was only slightly congested. Many of the peribronchial glands were enlarged, and upon section were seen to have undergone calcareous changes. The pleural cavities each contained about 150 c.c. of free light amber-colored fluid.

The heart was slightly enlarged. The right heart was normal and contained a large postmortem clot. The pulmonary artery and valve were normal. The left auricle was normal, but there was a marked thickening of the mitral valve. The musculature of the heart was thicker than normal and fairly firm. It was pale in appearance. The aortic valve was normal. The aorta showed many fatty and calcareous changes throughout its entire course, but more especially in the ascending and transverse arch.

The abdomen was opened and the intestines were distended with gas and the vessels were deeply injected. The appendix was *in situ* and normal in

appearance. The stomach epiploic vessels were also deeply injected. The stomach was of normal size and shape, but the mucous membrane showed a marked thickening and very deeply injected, streaked, red and yellow appearance. The pylorus was normal and easily admitted the tip of the index finger.

The liver was of normal size, smooth on the surface and of a dark congested appearance. The gall bladder and ducts were apparently normal.

The spleen was about normal size of a dark blue color and very soft. Upon section the follicles were easily visible and the surface had a congested appearance.

The right kidney, after some difficulty, was found in the normal position. The kidney was about the size of a kidney of a newborn infant. It presented a normal appearance externally, except for its size. Upon section, the cut edges everted and the cortex was well marked off from the medulla. The left kidney was somewhat larger than normal, but not so large as would have been expected with such an infantile right kidney. There was a dark purple swollen and softened area on the inner side of the kidney towards the lower pole, which only extended into the cortex of the kidney and appeared to be necrotic. Upon section, the cut edges everted, the cortex was thicker than normal, and not well marked off from the medulla. Throughout the kidney on the surface there were numerous whitish miliary areas resembling tuberculous areas or abscesses. These areas contained whitish soft caseous-like material.

The bladder was slightly distended and contained about 200 c.c. of clear yellow urine. The ureters and adrenals were apparently normal.

The posterior tibial artery was dissected from the right leg and foot. The radial and ulnar arteries were dissected from the right arm and hand. The middle finger of the right hand was amputated at the middle of the proximal phalanx.

DISCUSSION

The obvious interest of this case is the differentiation from the Raynaud syndrome. A gangrene with arteriosclerosis in an admitted syphilitic hardly comes under that diagnosis. A review of the rather voluminous literature of Raynaud's disease leads one to believe that the diagnosis is often made with scant logic. Reading the actual translation of Raynaud's original thesis and his "Newer Researches" one accepts the picture of a symmetrical peripheral gangrene due to a spasm of the vessels as the entity which the author wishes to differentiate. That this can not be logically done unless vascular disease is ruled out is obvious. Yet one is unpleasantly struck with the inadequate amount of pathologic certification of his twenty-two cases. Only six came to autopsy and the nature of the report in these is far from satisfactory. For instance, in Case 7, "The autopsy revealed nothing special." In Case 9, "Careful injection and dissection of the arteries was negative." This is the only case which Raynaud examined microscopically in person. In Case 17, "The arteries were adherent to the bone and closed at the line of demarcation." In Case 18, "The mitral was sclerotic and there was narrowing of the entire arterial system." In Case 19, "Both auriculoventricular orifices were narrowed by

sclerosis." In Case 22, "Both femoral arteries were remarkably shrunk and were not patulous on division with the knife."

The trump card for the vascular spasm theory in this syndrome is the observation of contraction and expansion of the retinal vessels in definite time relation with the peripheral symptoms. It is regrettable to discover that Raynaud observed this phenomenon definitely in but one of his twenty-two cases.

Clashing further with his own definition, he has included one case in which the gangrene alternated between the sides of the body and two in which it was unvaryingly unilateral.

Coming down to recent writers, one finds equal illogicality. Montgomery and Culver[1] report a case involving the left hand only with a relapse from "psychic shock." During the course of the disease there was a difference of blood pressure of 10 millimeters which gradually abated as a cure ensued. Differences of 10 millimeters are rather unconvincing.

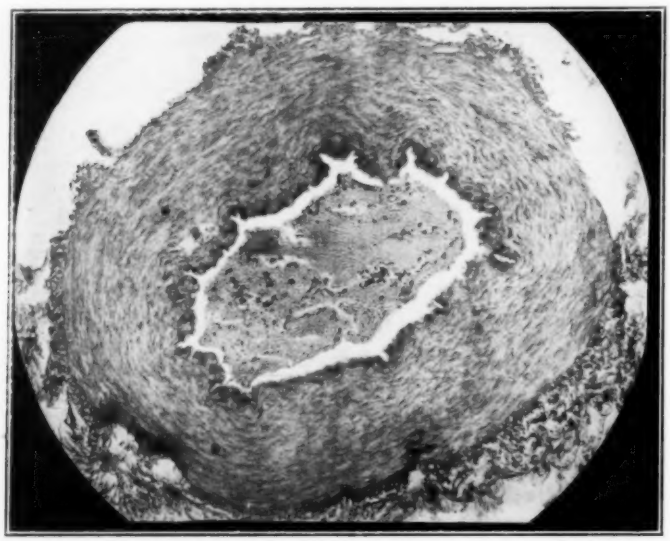

Fig. 1.

Osborne[2] reports a case of intermittent attacks of mental disturbance and mumbling in a known syphilitic who improved under salvarsan, and without any other evidence of vascular disease diagnoses "cerebral Raynaud."

In contrast there are many more carefully studied cases. Lisser[3] reports a case in a negro child with gonorrheal arthritis and a positive Wassermann reaction on the blood.

Glazer[4] reports a case in a child with hereditary syphilis which improved under mercury.

Lyle and Griewie[5] report a case in which autopsy revealed vascular sclerosis with small round-cell infiltration.

Simmons[6] reports a case not clinically syphilitic in which, on postmortem, slight thickening of the arteries without endarteritis was found.

Hoyne[7] reports the case of a child who in the course of forty-six days had

scarlatina, measles, and pertussis, developed symmetrical gangrene and died of bronchopneumonia. On autopsy streptococcus viridens and streptococcus hemolyticus were recovered from a femoral thrombus.

Buerger,[8] arguing for thromboangiitis obliterans as the cause of symmetrical peripheral gangrene, reports such a macroscopic diagnosis on vessels postmortem and after amputation. One case examined microscopically showed "typical lesions of thromboangiitis obliterans."

Clinically the case here reported personally would pass well as a Raynaud's syndrome. The palpable portions of the radial were soft, there was hemoglobinuria and the local picture was perfect. But the postmortem examination places it in another group. Macroscopically higher portions of the brachial arteries were sclerotic, and microscopically this is excellently brought out in Fig. 1. The sclerosis is diffuse and small round-cell infiltration is lacking so that syphilitic sclerosis can not be absolutely predicted.

Undoubtedly the diagnosis of Raynaud's disease as made lacks logical substantiation, and the case above is offered in behalf of differentiation.

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STUDIES ON DIPHTHERIA TOXIN*

By Lewis Davis, S.M., Detroit, Mich.

I.—HYDROGEN-ION CONCENTRATION AND TOXICOGENICITY DETERMINATIONS WITH BACT. DIPHTHERIÆ

HISTORICAL RESUME.—Some of the earliest workers with Bact. diphtheriæ have noted that the reaction of the culture medium has a decided influence on growth and toxin production with this organism. Roux and Yersin,[1] who appear to have been the first to extensively investigate this question, recommend the use of slightly alkaline bouillon, since they found that an acid reaction did not permit of strong toxin formation. Spronck[2] attributed this acid production to the presence of a variable quantity of glycogen and glucose, fermentable by Bact. diphtheriæ, in the meat used for the bouillon. To overcome this factor, he proposed the employment of decomposed beef or veal in which the putrefaction had reduced the content of these sugars to a minimum.

Park and Williams[3] found that with the beef used by them, the inhibitory action of the muscle sugar was neutralized by adding sufficient alkali to the bouillon (about 7 c.c. of normal soda solution per liter). They state "that an excessive amount of either acid or alkali prevented the development of toxin" and that the "type of growth of the bacilli and the rapidity and extent of the production of toxin depended more on the reaction of the bouillon than upon any other single factor."

Essentially the same results have been found by Madsen.[4] This investigator concludes that in the same bouillon, diphtheria cultures can develop in one of two directions, acid or alkaline, depending upon the initial degree of alkalinity. Confirming the results of Spronck, and Park and Williams, acid cultures were found by him to possess no toxicity, while, in general, the alkaline cultures are toxic, although no definite relationship exists between the degree of alkalinity and the amount of toxin produced.

In order to eliminate any action of the muscle sugar, Theobald Smith[5] proposed a peptone bouillon in which the beef infusion, previously reduced in acidity, was submitted to a preliminary fermentation overnight with B. coli, then sterilized and filtered. This would break up all carbohydrates, and give at once an alkaline growth with a constant, strong toxin. Addition of dextrose to such bouillon in quantities not exceeding 0.2 per cent, was found not only to be uninjurious, but actually led to a maximum accumulation of toxin, presumably by utilizing the available peptone to the best advantage. This method has not met with general favor, although Hitchens[6] reports good results from its use with veal bouillon. Lubenau[7] confirms the findings of Smith that no acid is formed in carbohydrate-free, nutrient bouillon, and also states that, as a rule, diphtheria bacilli produce alkali in such bouillon independent of the initial reaction. On the other hand, he finds that diphtheria-like organisms produce no appreciable amount of alkali, but leave a carbohydrate-free bouillon unchanged. In ordinary bouillon, which would contain carbohydrates, both diphtheria and diph-

*From the Research Laboratory of Parke, Davis & Co., Detroit, Mich.

theria-like organisms produce acid, regardless of the initial reaction of the bouillon.

Jacobsen,[8] in a critical review of the work of Madsen and of Lubenau, agrees that in sugar-free bouillon (Smith method) diphtheria cultures produce alkali at once. He also finds that, not only has dextrose an influence on the acid formation, but with a constant amount of glucose present in a bouillon, the amount of acid produced increases proportionately to the peptone content. Any culture of Bact. diphtheriæ in bouillon can, according to him, theoretically be considered as passing through the following stages: primary acid formation, reversal, alkali formation, secondary acid formation. This last stage which takes place only after a prolonged period of time (two to four months) has its origin in the neutralization products of the above mentioned reversal stage and is different from the primary acid formation which is attributed to the muscle sugar present.

A consideration of the work mentioned above, in the light of the modern conception of reaction based on hydrogen-ion concentration, brings up at once the limitations of results obtained by the usual titrametric methods. In fact, it is now generally conceded that there is a decided difference between the acid and alkaline values obtained by titration using litmus and phenolphthalein. Clark,[9] in an able discussion of the fallacy of titrating hot media, states, "It is needless to add that since titrations in the last analysis are based upon the attainment of a certain hydrogen-ion concentration as shown by the tint of an indicator, the titration of a medium at 90° to 100° C. furnishes data of no exact significance at ordinary incubation temperatures."

In connection with the development of a satisfactory bacteriologic peptone, I[10] have employed the production of a potent diphtheria toxin with a trial sample as one of the biologic tests. This required a study of the factors influencing growth and toxin formation in bouillon of constant composition and, as would be expected from the preceding resume, the hydrogen-ion concentration of the medium has been found to be of prime importance. The present investigation was undertaken to determine more accurately by means of the hydrogen electrode what reaction changes take place in the medium during the propagation of diphtheria toxin on a practical scale, and to note what relationship, if any, exists between toxicogenicity in a medium and hydrogen-ion concentration.

LABORATORY DATA

Methods of Experimentation.—Extensive experience has shown that plain bouillon gives uniformly satisfactory results in the production of toxin with Bact. diphtheriæ. Sugar media and carbohydrate-free broth (Smith method) have not proved of any advantage. In the study at hand, unless otherwise noted, the medium employed was plain bouillon, made up as given in the following:

To every liter of beef infusion prepared in the usual manner, 20 grams of peptone (Bacteriologic, Parke, Davis & Co.) and 5 grams of sodium chloride were added. Supplies of sterile beef infusion and of the other two ingredients from individual lots were retained in quantities sufficient for all of the experimentation so as to eliminate any variations of the bouillon components. The peptone and salt were first dissolved in the cold, then heated in a steamer for fifteen minutes to insure thorough solution.

The medium was now ready for preliminary adjustment to the desired hydrogen-ion concentrations which was done essentially as proposed by Clark and Lubs,[11] using the buffer solutions, standardized by the hydrogen electrode, and the indicators recommended by them. As will be later shown, the limiting concentration values for cultivation of the diphtheria bacillus are approximately $C_H^+ = 1 \times 10^{-6}$ ($P_H^+ = 6.0$) and $C_H^+ = 1 \times 10^{-9}$ ($P_H^+ = 9.0$), respectively, which reduces the standard buffer solutions necessary to the "acid potassium phosphate-sodium hydroxide" mixtures and the "boric acid-potassium chloride-sodium hydroxide" mixtures. "Brom cresol purple" and "brom thymol blue" were used as indicators for the media on the acid side; "brom thymol blue" for those concentrations around true neutrality, while in the alkaline zone, "phenol red," "cresol red," and "thymol blue" were employed. The various media were then neutralized essentially as described below for regular diphtheria toxin bouillon, the final ion concentration being determined electrometrically.

For the routine production of diphtheria toxin, in bouillon, hydrogen-ion concentrations around 1.0×10^{-8} ($P_H^+ = 8.0$) have been employed with excellent results. Such reaction values are rapidly obtained by taking, for the preliminary adjustment, 10 c.c. of the medium, after the first heating, diluting with about 40 c.c. of distilled water, and titrating against dilute (N/10) sodium hydroxide in the cold, using phenolphthalein as the indicator. A little experience will soon determine what shade of pink should be taken as the end point. The requisite amount of strong sodium hydroxide to entirely neutralize (10N is recommended), in accordance with the preceding titration, is now added slowly and with thorough mixing to the bouillon. The medium is next heated in flowing steam for 30 minutes, or boiled for five minutes, which brings down a heavy precipitate that rapidly settles.

The reaction of the clear, supernatant liquid, or a dilution of it with "conductivity" water, is now, for very accurate work, checked by the hydrogen electrode. Ordinarily, however, a colorimetric "check" by means of the simple comparator of Hurwitz, Meyer and Ostenberg[12] and using standardized mixtures (of $P_H^+ = 7.8$, 8.0, and 8.2) with "phenol red" will be found sufficiently accurate and more rapid. In my experience, the hydrogen-ion concentration of media prepared as above, falls within a range covered by $C_H^+ = 1.5 \times 10^{-8}$ to $C_H^+ = 7.0 \times 10^{-9}$ and may be used for diphtheria toxin production without any further adjustment. The bouillon is now filtered hot, distributed as desired, and sterilized. All of the media used in the present investigation have been sterilized once for 20 minutes at 115° C. (15 pounds steam pressure).

Comparative toxicity determinations have shown that more satisfactory results are obtained by using larger flasks for cultivation of Bact. diphtheriæ than the same proportionate amount of bouillon distributed in smaller containers. Accordingly, 3,000 c.c. of the test medium in six liter flasks were employed and inoculations made in each case, with 24 hour "starters" containing 30 c.c. of culture grown in a 250 c.c. flask to accustom the organism to the medium. After culturing for the desired length of time at 37° C., the purity of the growth was checked, the samples preserved with 0.4 per cent cresols and filtered through unglazed porcelain.

In the preliminary experimentation on the ion concentration changes pro-

duced during growth of Bact. diphtheriæ, use was made of the large flasks fitted with special syphons, so that samples could be removed aseptically from time to time as desired. Control flasks, without syphons, showed that this procedure so interfered with the growth through disturbance of pellicle formation as to entirely vitiate the value of any results obtained. A sufficient number of flasks has accordingly been employed to enable triplicate determinations of any desired value. In practically every case it has been found possible to obtain duplicate hydrogen-ion concentration and toxicity results which checked within practical limits. The toxicity valuations, made in association with my colleague, T. Ohno, were, unless otherwise noted, determined for L_+ dose on samples in duplicate.

The electrometric determinations of hydrogen-ion concentration were made with the chain:

Calomel electrode (*N* KCl) — saturated KCl — test medium — Pt Electrode — (H_2) at 23° C. The complete "set up" employed is shown in Fig. 1. *C* is a "normal" calomel electrode which dipped into the small vessel *D* containing saturated KCl to reduce to a minimum, the contact potentials between the *N* KCl and the liquid under examination. Connection between *D* and the test fluid in electrode vessel *A* was maintained by a wick saturated with KCl, passing through a small glass syphon tube *H*. Hydrogen electrodes (*B*) of the type described by Bovie,[13] were employed, supplied with purified hydrogen, generated from the electrolysis of NaOH with nickel electrodes. The temperature of the liquid in *A* was determined by a thermometer accurate to ± 0.1° C., and a similar thermometer was mounted near the calomel electrode *C*.

The hydrogen-ion concentration was read directly by means of the "ionometer" (*E*) described by Bartell.[14] The known potential derived from a Weston Standard Cell (*S*) (calibrated by the Bureau of Standards) was first brought into the circuit through the double-pole, double-throw switch *F* after which, sufficient resistance (4821 ohms) was thrown in to compensate for the calomel electrode employed. With the pointer arms of the logarithmic and concentration coils set at the proper values, (in this case 3.0 and 10^{-13} respectively) the external resistances, to compensate for the storage battery, were now adjusted until no deflection was noted in the galvanometer (*G*), which indicated that the instrument was in balance for direct reading. The resistance values thus obtained were found to remain practically constant for several hours with a good accumulator.

By reversing *F* the unknown cell consisting of hydrogen electrode and calomel was next connected in; the other resistances remaining the same, and readings could now be obtained directly in terms of hydrogen-ion concentration by manipulating the concentration and logarithmic coils until the galvanometer showed a balance. The ionometer and galvanometer were mounted so as to insure proper insulation from stray currents and freed as much as possible from tremors. During all of the measurements, the room temperature was such that no difficulty was experienced in keeping the thermometer practically constant at 23° C.

Experimental Protocols.—Before making a study of the toxicogenic and hydrogen-ion concentration changes produced by the growth of Bact. diphtheriæ in bouillon, the question arose as to what is the optimal zone of ion concentra-

tion for such metabolic activities. This made it necessary to determine within what limits of acidity and alkalinity, a known, toxicogenic culture of Bact. diphtheriæ can produce toxin of prescribed strength. For this purpose there were made up, as already described, lots of bouillon varying in reaction and sufficient in amount to permit of triplicate determination with large flasks at each ion concentration. The flasks were now inoculated with "starters" of a Bact. diphtheriæ

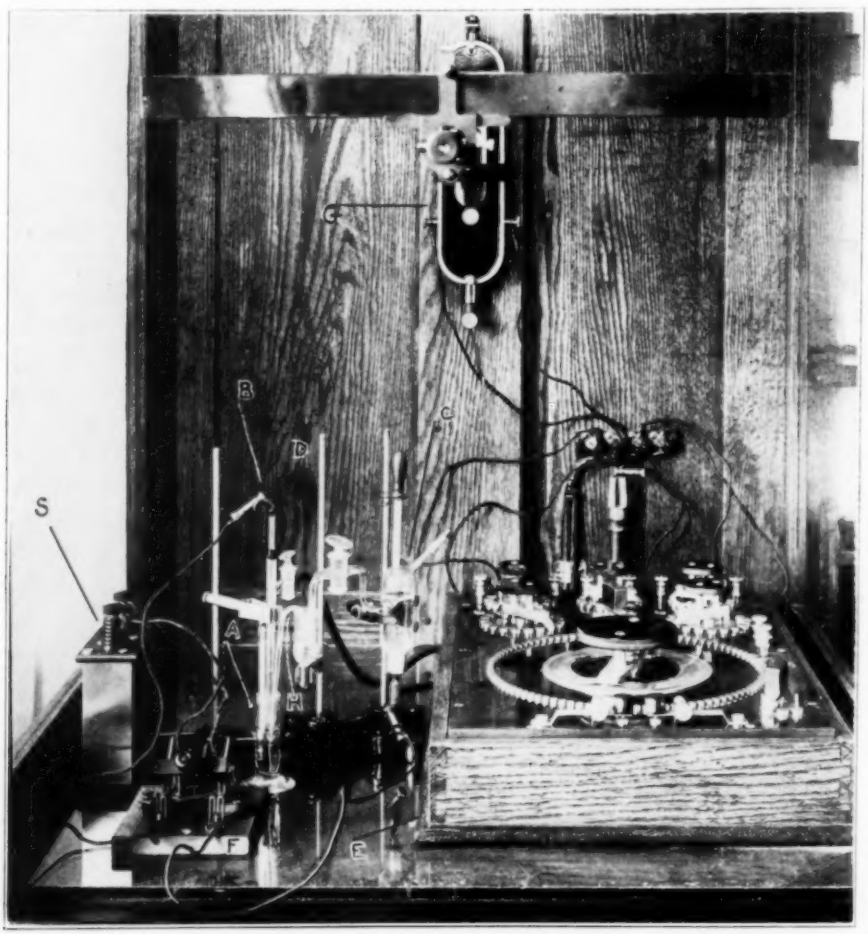

Fig. 1.

culture (No. 036) originally obtained from W. H. Park and capable of elaborating a toxin in standard bouillon of which one L+ dose is less than 0.25 c.c. Cultivation of each set was carried on at 37° C. for two weeks and at the end of this period samples were removed from each flask for determination of the final hydrogen-ion concentration. The remainder of the bouillon was then preserved and set aside for toxin estimation. The results obtained are summarized in Table I.

Inspection of the data tabulated in Table I shows that the optimal zone for metabolic activity of Bact. diphtheriæ is in the alkaline region. While good *growth* of the organism appears to be possible within hydrogen-ion concentration limits

TABLE I

THE INFLUENCE OF VARYING H-ION CONCENTRATIONS ON GROWTH AND TOXICOGENICITY OF BACT. DIPHTHERIÆ

INITIAL C_H^+	CHARACTER OF GROWTH	FINAL C_H^+ (2 WEEKS)	TOXICITY L^+ DOSE
6.5×10^{-6}	Very scant	4.2×10^{-6}	More than 2.0 c.c.
1.0×10^{-6}	Moderate	7.0×10^{-8}	Between 1.5 and 2 c.c.
7.5×10^{-7}	Heavy	2.1×10^{-8}	1.0 c.c.
1.0×10^{-7}	"	2.0×10^{-8}	0.7 c.c.
7.0×10^{-8}	Very heavy	1.8×10^{-8}	Less than 0.25 c.c.
3.6×10^{-8}	" "	1.6×10^{-8}	" " 0.25 "
2.0×10^{-8}	" "	9.7×10^{-9}	" " 0.25 "
1.0×10^{-8}	" "	7.6×10^{-9}	" " 0.25 "
8.5×10^{-9}	" "	7.2×10^{-9}	" " 0.25 "
7.0×10^{-9}	" "	6.9×10^{-9}	" " 0.25 "
5.0×10^{-9}	" "	5.2×10^{-9}	" " 0.25 "
2.3×10^{-9}	" "	3.0×10^{-9}	" " 0.35 "
1.1×10^{-9}	Moderate	2.3×10^{-9}	Between 0.50 and 0.6 c.c.
8.4×10^{-10}	"	4.1×10^{-9}	" 0.75 " 1.00 "
7.0×10^{-10}	Scant	1.0×10^{-8}	" 1.5 " 2.0 "
5.3×10^{-10}	Very scant	7.5×10^{-10}	More than 2.0 c.c.

ranging from about $C_H^+ = 10 \times 10^{-6}$ to about $C_H^+ = 8.4 \times 10^{-10}$, maximum production of toxin seems to occur only where the reaction of the bouillon falls in a concentration range from about $C_H^+ = 7.0 \times 10^{-8}$ to about $C_H^+ = 5.0 \times 10^{-9}$. Anti-

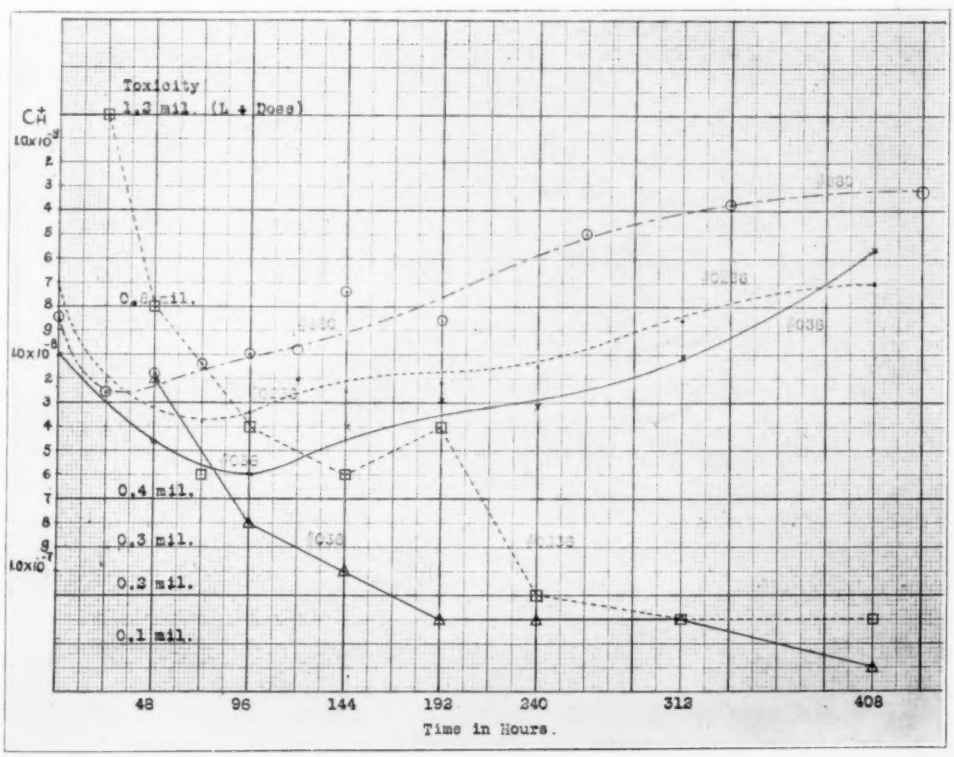

Fig. 2.

septic action, as evidenced by scant growth, is apparently exerted on the acid side around $C_H^+ = 6.5 \times 10^{-6}$ while on the alkaline side, it does not come into consideration until about $C_H^+ = 5.3 \times 10^{-10}$ is reached.

With the foregoing results at hand, experimentation was now undertaken to determine the changes in reaction and toxin production which take place in plain bouillon during the growth of Bact. diphtheriæ cultures under optimum conditions. In addition to the Park strain (No. 036) employed above for the antiseptic value test, another toxicogenic strain (No. 0236) originally obtained from McFarland, and an avirulent nontoxicogenic strain (No. 880) isolated by H. C. Ward of this laboratory were studied. The preparation of the bouillon for this purpose and the inoculation technic have been detailed in the preceding section. The accompanying curves shown in Fig. 2 have been plotted using the data obtained with the three cultures, and tabulated in Table II.

TABLE II

CHANGES IN H-ION CONCENTRATION AND TOXICOGENICITY DURING GROWTH OF BACT. DIPHTHERIÆ IN BOUILLON

(Park) Strain No. 036			*(McFarland) Strain No. 0236*			*(Avirulent) Strain No. 880*		
TIME IN HOURS	C_H^+	TOXICITY L^+ DOSE	TIME IN HOURS	C_H^+	TOXICITY L^+ DOSE	TIME IN HOURS	C_H^+	TOXICITY M.F. DOSE
0	1.0×10^{-8}	—	0	7.0×10^{-9}	—	0	8.5×10^{-9}	—
			24	2.1×10^{-8}	1.0 c.c.	24	2.3×10^{-8}	No reaction
48	4.6×10^{-8}	0.65 c.c.	48	3.2×10^{-8}	0.8 "	48	1.9×10^{-8}	2 c.c.
			72	3.8×10^{-8}	0.45 "	72	1.3×10^{-8}	
96	6.0×10^{-8}	0.35 "	96	3.4×10^{-8}	0.55 "	96	1.1×10^{-8}	
			120	2.1×10^{-8}	0.35 "	120	9.5×10^{-9}	No reaction
144	4.0×10^{-8}	0.25 "	144	2.5×10^{-8}	0.45 "	144	7.4×10^{-9}	2 c.c.
192	2.9×10^{-8}	0.15 "	192	2.3×10^{-8}	0.55 "	192	8.3×10^{-9}	
240	3.0×10^{-8}	0.15 "	240	1.6×10^{-8}	0.15 "			
						264	5.5×10^{-9}	
312	1.2×10^{-8}	0.15 "	312	8.7×10^{-9}	0.15 "			
						336	3.8×10^{-9}	Necrosis 2 c.c.
408	4.7×10^{-9}	0.05 "	408	7.0×10^{-9}	0.15 "			
						432	3.2×10^{-9}	Necrosis 2 c.c.
			528	5.0×10^{-9}	0.15 "			

Comparison of the hydrogen-ion concentration curves of Fig. 2 shows a decided similarity with all three of the strains examined. There is, at first, a small production of acid, as shown by an increase in the concentration of hydrogen ions, which soon reaches a maximum, the amount of acid produced varying with the organism. The Park strain (No. 036) gives the maximum amount of acid, the other toxicogenic strain (McFarland No. 0236) produces nearly as much, while the avirulent strain develops only about one-half of the amount.

Inspection of Fig. 2 indicates that the slope of the acid production curves is very nearly the same for the three strains, so that the total increase in hydrogen-ion concentration directly depends on the length of time the organism undergoes acid fermentation. Strain No. 036 required four days to reach a maximum value, No. 0236 apparently had attained this point in 72 hours while No. 880 seems to have begun an alkaline fermentation after the first day. In fact, it

will be noted that all three strains show a reversal, followed by alkaline fermentation which apparently continues until an antiseptic hydrogen-ion concentration is reached.

By way of obtaining some information as to the source of the acid production in plain bouillon, two sets of media were prepared as already directed. One set consisted of the beef infusion used in the preceding experimentation with 0.5 per cent sodium chloride, and the other had 2 per cent of the peptone and 0.5 per cent of the sodium chloride. Both sets were inoculated with starters of the Park strain (No. 036) and the hydrogen-ion concentration changes occurring in each during growth were now studied in accordance with the technic already employed. The results are shown in Table III.

TABLE III

HYDROGEN-ION CONCENTRATION CHANGES DURING GROWTH OF BACT. DIPHTHERIÆ IN STRAIGHT BEEF INFUSION AND 2% PEPTONE SOLUTION

Beef Infusion			*2% Peptone Solution*		
TIME IN HOURS	C_H^+	TOXICITY M.F. DOSE	TIME IN HOURS	C_H^+	TOXICITY M.F. DOSE
0	8.5×10^{-9}		0	7.5×10^{-9}	
24	9.4×10^{-9}	No reaction 2 c.c.	24	8.4×10^{-9}	No reaction 2 c.c.
48	1.3×10^{-8}	" "	48	1.0×10^{-8}	" "
96	2.7×10^{-8}	" "	96	8.7×10^{-9}	" "
168	2.7×10^{-8}	" "	168	7.0×10^{-9}	Slight reaction 2 c.c.
240	2.6×10^{-8}	" "	240	6.5×10^{-9}	" "
408	2.9×10^{-8}	" "	408	6.1×10^{-9}	No reaction 2 c.c.

It is readily apparent from the above data, that both components of plain bouillon permit of acid fermentation by Bact. diphtheriæ. As was expected, the growth in straight infusion was scant, while that in the 2 per cent peptone solution (+ salt) appeared only about half as heavy as in regular bouillon. Table III shows that in plain beef infusion, the diphtheria bacillus produces a small amount of acid which reaches a maximum in about four days, after which the hydrogen-ion concentration remains practically constant. In the peptone solution, there is also an initial acid production increasing to about the third day, but then followed by a reversal and steady decrease in hydrogen-ion concentration as in bouillon. Estimation of the amounts of acid produced in each case indicates that the sum total of both sets very nearly approximates the initial H-ion increase during growth in plain bouillon, as given in Fig. 2.

The toxicity values for No. 036 show a steady increase until the eighth day. Five days later, the value appears to be constant, but increases in toxicity at the end of seventeen days. The toxin development in the case of No. 0236 is irregular, fluctuating up to the eighth day, after which there is a rapid increase in toxicity, reaching a maximum on the thirteenth day and remaining constant at this value to the end of the experiment. It is interesting to note that the avirulent strain (No. 880) produces no toxin at first (2 c.c. dose), but sufficient is developed at the end of two weeks to cause necrosis at the site of injection with the same dose. That it is actually diphtheria toxin which is

produced, is shown by the fact that addition of antitoxin (as in testing by L+ dose) causes neutralizaton and no necrosis.

DISCUSSION

As has been found true in the case of several other organisms, notably in the colon and streptococcus groups (Michaelis and Marcora,[15] Ayers,[16] Itano[17]), the hydrogen-ion concentration of the medium, from the data presented above, is seen to have a decided influence on the metabolism of Bact. diphtheriæ. While there is relatively a wide range, from about $C_H^+ = 1.0 \times 10^{-6}$ to about $C_H^+ = 8.4 \times 10^{-10}$, in which good growth can take place, maximal elaboration of toxin occurs only in a small zone of hydrogen-ion concentration, covered by $C_H^+ = 7.0 \times 10^{-8}$ to $C_H^+ = 5.0 \times 10^{-9}$. The upper limits of alkalinity very closely approximate in value those obtained by the author for the H-ion concentration of antidiphtheric serum (equine) and the lower is very near the $C_H^+ =$ value given by McClendon and Magoon[18] for blood.

It is interesting to note from Table I that Bact. diphtheriæ can tolerate a much greater concentration of hydroxyl ions (stronger alkaline reaction) than acid ions before a value inhibitory to growth is encountered. In all probability, the antiseptic C_H^+ values obtained with the Park strain (6.5×10^{-6} and 5.3×10^{-10}) are of general application to Bact. diphtheriæ since "starters" of the McFarland Strain (No. 0236) fail to develop at $C_H^+ = 6.5 \times 10^{-6}$ and give only very scant growth in a medium reading $C_H^+ = 5.0 \times 10^{-10}$. No growth at all can be obtained with the avirulent strain at either of the above mentioned hydrogen-ion concentrations.

Confirming, in a general way, the results obtained by other investigators, the curves plotted in Fig. 2 show that B. diphtheriæ, when cultivated in plain bouillon undergoes an initial acid fermentation. Contrary to what might be supposed from the introductory review, the total increase in hydrogen-ion concentration, even with the most vigorous strain (Park, No. 036) is relatively small (from about $C_H^+ = 1.0 \times 10^{-8}$ to $C_H^+ = 6.0 \times 10^{-8}$), and appears to be due to an acid fermentation of some constituent, very likely carbohydrate in nature, in both the beef infusion and the peptone. It should also be noted that even at the point of maximum acid development, the reaction is definitely alkaline.

The results given in Table I strongly indicate that an actual acid reaction ($C_H^+ = 1.0 \times 10^{-7} =$ neutral) has a destructive action on diphtheria toxin. From the amount of acid produced by both of the above toxicogenic strains, it would seem that where the initial C_H^+ of the medium is appreciably greater than 7.0×10^{-8}, the increase in hydrogen-ion concentration resulting from growth would be sufficient to produce a final acid reaction destructive to the toxin. This is actually found to be the case. Table I shows that where the initial reaction of the bouillon is $C_H^+ = 7.0 \times 10^{-8}$, a strong toxin with L+ dose of less than 0.25 c.c. is obtained. On the other hand, a slight increase in the initial reaction to $C_H^+ = 1.0 \times 10^{-7}$ is sufficient to make the final potency drop to an L+ dose of about 0.7 c.c.

The total amount of acid produced appears to be a specific property of each individual strain. The avirulent strain develops only about half as much acid as do the toxicogenic strains, the increase in hydrogen-ion concentration reaching a maximum soon after the first day in the former case. It may be probable

that this diminished capacity for acid fermentation is a general property of avirulent strains and is possibly an index of decreased metabolic activity.

The data given in Tables II and III further show that the natural course pursued by Bact. diphtheriæ growing in plain bouillon is in the direction of increased alkali production. This decrease in hydrogen-ion concentration takes place steadily after the initial acid fermentation and reversal, and apparently continues until a limiting concentration is attained.

It is obvious from the curves plotted in Fig. II that in the normal development of Bact. diphtheriæ in bouillon, the organism may produce the same hydrogen-ion concentration at two different intervals, which represent wide variations in potency. Other than the fact that it is necessary to have the initial reaction of the bouillon within the alkaline limits above mentioned, and that potent toxin will have an alkaline reaction, the experimental data indicate that there is no direct relationship during growth between the hydrogen-ion concentration of the medium and the production of toxin.

In conclusion, I desire to express my most sincere thanks to Professor F. E. Bartell of the University of Michigan for valued advice on the determination of hydrogen-ion concentration.

CONCLUSIONS

1. Toxin of maximum potency is produced in bouillon by Bact. diphtheriæ only when the initial reaction falls within a certain zone of alkalinity, included within the hydrogen-ion concentration limits of about 7.0×10^{-8} to about 5.0×10^{-9}. Luxuriant growth of the organism appears to be possible where the reaction of the bouillon ranges from about $C_H^+ = 1.0 \times 10^{-6}$ to about $C_H^+ = 8.4 \times 10^{-10}$.

2. When cultivated in plain bouillon under optimal conditions, Bact. diphtheriæ undergoes an initial increase in hydrogen-ion concentration. This is soon followed by a steady decrease until apparently a limiting alkaline reaction is attained. The total acid produced is relatively small and seems to vary in amount with each individual strain. Toxicogenic strains appeared to develop more acid than an avirulent strain. The initial increase in hydrogen-ions is due to fermentation of some constituent in both peptone and beef infusion.

3. No direct relationship can be found between the hydrogen-ion concentration of the medium and toxicity during the growth of Bact. diphtheriæ.

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LABORATORY METHODS

NOTE ON THE COLLOID CHEMISTRY OF FEHLING'S SUGAR TEST*

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As familiarly known, especially by the clinicians, the reduction of Fehling's alkaline copper solution to cuprous oxide by sugars and certain other reducing materials presents variations, the nature of which is not yet fully understood. In testing the urine of diabetics for dextrose, for example, it is usually expected that the presence of sugar will betray itself through a reduction of the copper salt to a bright red precipitate of copper oxide. It frequently happens, however, that the reduction does not yield this bright red result, but a more orange, or distinctly yellow precipitate; while, under certain circumstances, only a greenish discoloration of the originally bright blue solution is obtained, from which, at the best, a light greenish or dirty yellow precipitate may settle out in the course of many days.

The explanations which have been given of these findings are different with different authors, but they are, for the most part, chemical in nature. The appearance of the green color, with little tendency to form a precipitate, is often regarded as entirely questionable evidence of the presence of dextrose (or other reducing material). The yellow precipitate is usually accepted as clear proof for the presence of a reducing body (like dextrose) but this yellow substance is not considered identical chemically with the red cuprous oxide. Many, for example, hold the yellow precipitate to be a hydrated form of the copper oxide.

Because of this confusion regarding the nature and the interpretation of the reaction, we decided to examine it from a colloid-chemical point of view, for we felt in advance that the explanation of what was really at the bottom of these apparent differences in end results was more likely to be found in these regions than in the more commonly studied ones of pure chemistry. We felt that *Fehling's reduction test, under different circumstances, does not yield chemically different copper oxides, but one and the same copper oxide in different degrees of subdivision* (possessed of different degrees of dispersion).

That one and the same chemical compound (as a metal, a sulphide or an oxide) may, in the colloid state, show different colors has long been known to different workers.[1] It has, however, remained for Wolfgang Ostwald[2] to recognize that these color variations tend, on the whole to follow a general order and that this order is coordinate with the size of the dispersed particles. The most highly dispersed particles of a given substance are likely to be yellow; as their size increases, they become orange, then red, and finally violet, blue, or black.

*From the Eichberg Laboratory of Physiology in the University of Cincinnati.

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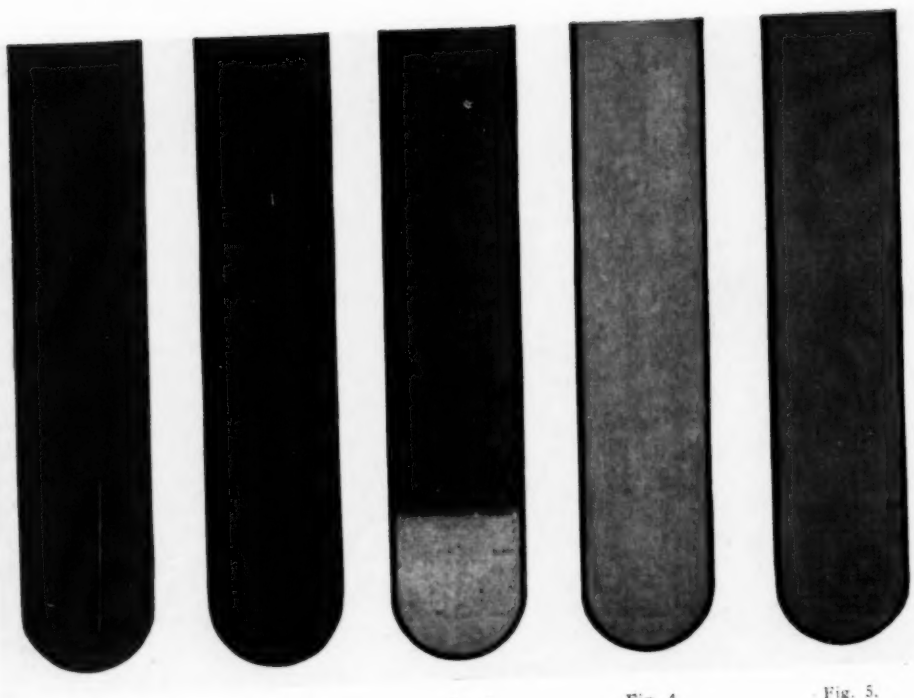

Fig. 1. Fig. 2. Fig. 3. Fig. 4. Fig. 5.

When the ordinary, brilliantly blue Fehling's solution is mixed with a little dextrose solution or some diabetic urine and the mixture is not boiled, as ordinarily, but is simply allowed to stand a number of hours at room temperature, a series of such color changes as are represented in Figs. 1 to 5 may be observed. The originally clear Fehling's solution early loses its brilliancy and assumes the more opaque blue green color of Fig. 1. It soon passes from this to the leaf green of Fig. 2 and then to the yellow green of Fig. 3. At this time more or less yellow precipitate may separate out. Later, not only this yellow precipitate, but the supernatant liquid as well become yellow orange, then bril-

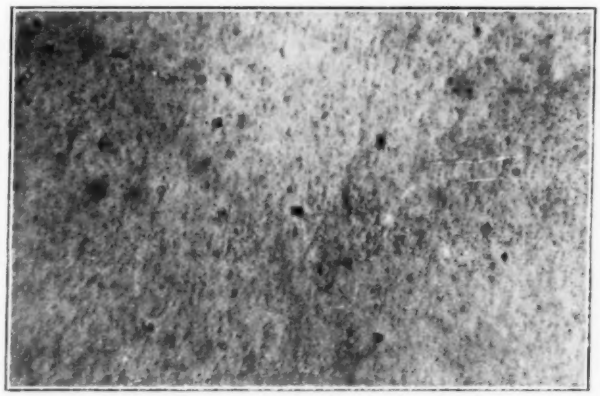

Fig. 6.

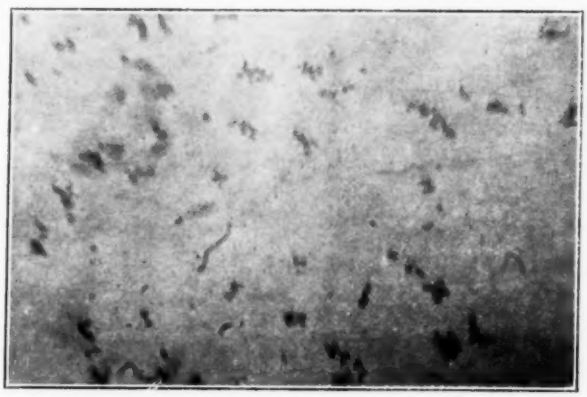

Fig. 7.

liantly orange as in Fig. 4 and finally the bright red shown both by the solution and precipitate of Fig. 5.

It is of interest now to follow the microscopic appearances which are coincident with these macroscopic optical changes. This may be done by examining drops of fluid from different tubes as the different colors are developed, or the whole series may be observed in one and the same drop as the reduction progresses under the microscope. Examined microscopically or ultramicroscopically, the original Fehling's solution is optically homogeneous. If a drop of the

fluid from such a less brilliant tube as is shown in Fig. 1 is examined, one early becomes conscious of a vague blurring in the microscopic field. By waiting a little, this is seen to be followed by a brilliant lighting-up of the field through vast numbers of actively motile particles. A photograph taken about this time yields Fig. 6. We may now examine drops of fluid from the yellow, orange, or red tubes or we may simply watch the original preparation under the microscope as this passes, successively, through these same color changes. As shown in Figs. 7 and 8, the particles gradually grow in size so that when they appear distinctly yellow and start to precipitate as in Fig. 3, the microscopic picture shown in Fig. 7 is observed. When the brilliant red precipitate is reached, the microscope reveals particles of the size shown in Fig. 8.

These observations show, therefore, that *the different colors observed in the reduction of Fehling's solution by dextrose (or other reducing substances) are nothing more than color changes coincident with a gradual increase in the size of copper oxide particles.*

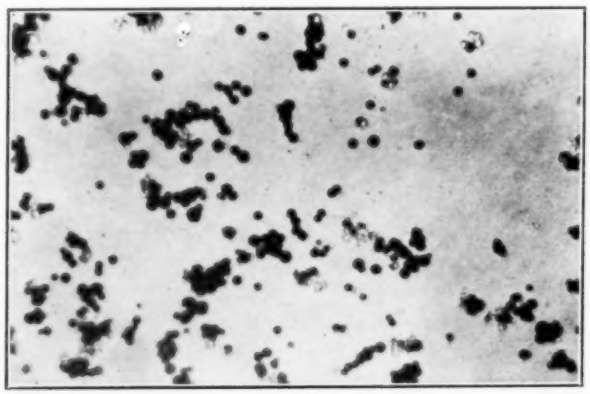

Fig. 8.

To the practical chemist who, like the clinician, uses Fehling's test in order to determine qualitatively or quantitatively the presence of reducing substances in any fluid (like urine), the simple observations detailed in this paper are not only useful, but they make clear the meaning of several things which have previously been ambiguous.

These observations show that any of the positive reactions here described (whether they yield merely a dirty green solution, a yellow or orange precipitate, or the brilliantly red precipitate so generally sought) indicates equally well the presence of a reducing substance. A dirty pea-green solution, in other words, means just as much from a qualitative point of view as does a red precipitate. Most textbooks would have it that this is not the case, or, if they admit that such a reaction is important qualitatively, they hold that it means the presence of but little reducing material. It is of interest, therefore, to emphasize that in the case of dextrose, for example, the presence of much of this material is quite as likely, in fact more likely, other things being equal, to yield merely a greenish discoloration or a yellow precipitate than is a lesser amount. In fact, to

insure getting the red precipitate, one must in all instances use *little* enough of the reducing material. Generally speaking, amounts of dextrose too small to reduce all the copper salt present will more definitely yield a red or an orange precipitate than larger amounts.

This fact, as well as certain others to be discussed immediately will, of course, be readily intelligible to the colloid chemist. With much sugar present, the number of points at which the copper salt is attacked and reduced will evidently be much larger than when less sugar is added. All the available copper salt for further growth of the particles will, therefore, have been exhausted when the copper oxide particles are still small, wherefore the presence of too much reducing substance is more likely to yield only the allegedly ambiguous end reaction than when less is used.

Another factor must, however, be considered as active in determining what the end result will be. Obviously, if the reduced copper oxide can be stabilized anywhere in its progress from the finely divided material to the coarse red, then one of the intermediate colors is obtained. As is also familiar to the colloid chemist, such stabilization of a finely divided suspension colloid is commonly brought about through the presence in the reaction mixture of various hydrophilic (lyophilic) colloids.

The truth of this general conclusion may be tested, not only experimentally with the pure materials of the chemical laboratory, but it is done daily in the clinicochemical experience of the laboratory worker in medicine. If to the dextrose solution used to produce such a series of color reactions as is shown in Figs. 1 to 5 we add some mucin, some acacia, some gelatin, a little egg-white or some other hydrophilic colloid, the rate at which the various colors is obtained is much delayed. In fact, if the concentrations are chosen properly, only dirty green or yellowish green reductions may be obtained, no matter how much copper oxide reducing substance may be present. The copper oxide may, in other words, be stabilized in any of its various states of subdivision.

What has been written above might seem at first sight not to harmonize with the practical fact, well known to the clinicians, that the urine of a severe diabetic will more often give a more perfect (red) reduction of Fehling's solution than will the urine from a milder diabetic or that from a normal individual in whom an alimentary glycosuria has been produced by excessive consumption of carbohydrate. The reasons for this are to be found in the fact that a severe diabetic with high dextrose elimination is usually also the victim of a polyuria. His urine while high in reducing substance (and consequently by itself inclined to yield a green or yellow result) is, therefore, relatively poor in those protective substances which give normal (nonpolyuric) urine its restraining powers for the stabilization of the copper oxide particles when these are still small. Conversely, the urine of a more nearly normal individual (containing, for example, but little dextrose following an excessive carbohydrate ration) will, it is true, contain but little reducing substance, but what makes for the stabilization of the copper oxide in its finely divided form is the large content of protective materials present.

The scientific basis of the old trick of diluting heavily the material to be examined whenever such questionable reductions are obtained is easily seen.

Dilution not only dilutes the highly concentrated reducing substance, but, more important, it dilutes the stabilizing colloids to a point where their powers in this direction are largely lost.

To these factors of the concentration of the reducing substance itself and the concentration of the various protective colloids which may be present comes a third factor which determines the nature of the end result. When dextrose, for example, is treated with an alkali, a series of degradation products is formed, as is well known, and it is these which in their turn are the elements directly responsible for the reduction of the copper salt. In the formation of these degradation products, however, some are produced which are in themselves colloid and hydrophilic in type. When, therefore, too much of a solution containing dextrose or any similar reducing substance is added to Fehling's solution, there appears the danger of getting intermediate colloid substances which in themselves tend to stabilize the copper oxide before it has attained the coarse dimensions necessary to yield an orange or red precipitate.

A final word must be added regarding the effects of temperature in the production of the red precipitate in a reducing Fehling's solution. Reduction at high temperature (as in the usual procedure) is more likely to yield the colloid types of reduction products than lower ones (like room temperatures). A urine containing an amount of reducing body which upon boiling yields only a dirty green solution, will show a bright red precipitate if the same proportions of urine and Fehling's solution are simply mixed and set aside at room temperature for twenty-four hours. The reasons for such improvement are probably several, but the most important would seem to be the initiation of the reduction at fewer points under the latter circumstances, while, because of the greater time element, these fewer points would then be given better opportunity to grow to the sizes characteristic of the red precipitate.

ADDENDUM

Since writing the above and since the appearance of a preliminary note[3] discussing this subject, Stanley Benedict has called our attention to the fact that many of the points made in our communication have been previously recognized by Hugh MacLean.[4] MacLean, in 1906, questioned the chemical nature of the differences in the results obtained upon reducing Fehling's solution and brought forth evidence which he felt indicated that these differences were merely physical. While his proofs have not been accepted as final by Benedict, we think, in the light of the additional facts detailed above and the clearer notions now available than then regarding the essential nature of the colloid state, that MacLean's general conclusions may be accepted with entire safety.

Failure to get a red precipitate MacLean holds to be due to something which keeps the cuprous oxide "in solution." If the word "solution" is not interpreted too strictly, but in the way in which it was used a decade ago when we still felt free to speak of liquid colloid suspensions as "solutions," then no objection must be raised to this contention of the author. As a matter of fact, in developing his theme he shows that what he means by "solution" *is* a suspension of particles of different sizes. He demonstrates these differences by showing that the green, the yellow, the orange, and the red liquids, or pre-

cipitates, pass with increasing difficulty through filters of known porosity. But this filtration method as we now know constitutes one of the very schemes employed by colloid chemists in order to determine (somewhat roughly) the size of particles in colloid suspensions. Finally, in trying to say what it is, in urine for example, which keeps the cuprous oxide "in solution" (or, in more modern terms, in a state of high dispersion) he fixes first attention upon the presence of creatinine. MacLean holds that this substance (rather specifically it would seem from the contentions of the author) keeps the cuprous oxide "in solution." Considering the fact that any hydrophilic colloid which is not dehydrated by the conditions of the experiment will retard or prevent the development of the cuprous oxide to its red form, a specific action is probably not to be assigned to the creatinine. As MacLean himself points out, the creatinine by itself "discolors" but does not reduce Fehling's solution. By removing some of the available copper, addition of the creatinine, therefore, substitutes for the ordinary mixture in which the amount of copper salt exceeds the amount of the reducing body, one in which the opposite conditions obtain. This, therefore, by itself, tends to give the yellow or green result. Beyond this the creatinine may form new and colloid compounds with some of the constituents of the Fehling's solution from which a general colloid protective action as detained above would then result.

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ON THE COLLOID-CHEMICAL MIMICRY OF CERTAIN ENZYMATIC REACTIONS*

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IN the course of experiments in which Fehling's solution was reduced by different substances, we encountered a series of reactions which are so strikingly analogous to certain reactions observable in living protoplasm that we have used these reactions for class demonstration purposes. While various aspects of the phenomena to be described are, since Bredig's experiments, well known to physical chemists, their sum total is somewhat new, and having proved of teaching value in physiologic discussion we publish them here with the feeling that they may possibly prove of interest to a wider circle of laboratory workers.

As well known, formaldehyde will reduce at room temperatures a Fehling's

*From the Eichberg Laboratory of Physiology in the University of Cincinnati.

solution not only to the ordinary cuprous oxide, but to the metallic copper. As the copper begins to be formed it comes down in a finely divided state, in other words, in colloid form. As soon, however, as this colloid copper appears, a second reaction ensues. The metallic copper begins to act upon the formaldehyde and to decompose this with the liberation of a gas. The gas is hydrogen. When this experiment is done at ordinary room temperatures, when the Fehling's solution and the formaldehyde are first mixed (in the proportion of 10 c.c. of 40 per cent formaldehyde to 90 c.c. of Fehling's solution), nothing apparently happens; but, after some hours the bright blue color becomes less transparent as a consequence of the gradual formation of the metallic copper, and, as this ensues, tiny bubbles arise throughout the fluid so that the whole mixture becomes distinctly effervescent. This process continues until all the formaldehyde has been decomposed or until all the blue color disappears and nothing is left behind but a spongy copper, from which there still rise occasional bubbles of liberated gas.

When biologic terms are applied to the set of reactions here described, we may say that, from the mixing together of a metallic salt, an alkali and a simple carbohydrate, we observe, first, the production of an enzyme (the colloid copper). In other words, there results from mixing together a series of very simple "dead" substances the production of that most characteristic of all the substances which characterize "living" matter. Looked at another way, the Fehling's solution represents a reaction mixture which is "injured," "poisoned" or destroyed by the formaldehyde. But against this "poison" or "toxin" the reaction mixture produces an "antitoxin" (the reduced copper), which in its turn decomposes the formaldehyde and so retards or prevents its further injurious action upon the Fehling's solution.

The analogy to biologic reactions is further heightened when it is pointed out that the presence of various substances will not only prevent the formation of the enzyme (the reduced copper), but will inhibit its action after formation. Potassium cyanide (which according to the classical findings of Geppert is particularly active in this regard in biological reactions, and according to the findings of Bredig, in the case of the colloid inorganic "ferments") shows marked action upon the decomposition of Fehling's solution by formaldehyde. Whether the cyanide be added to an original mixture of Fehling's solution and formaldehyde, or whether it be added after the copper reduction and the secondary decomposition of the formaldehyde have been well established, presence of the cyanide inhibits or stops entirely further decomposition in the reaction mixture.

The decomposition of formaldehyde under the influence of colloid copper with the production of hydrogen may also be used to illustrate much of what is considered fundamental in respiration. As emphasized in the classic views of Hoppe-Seyler, the production of nascent hydrogen is held to be essential in the chemistry of biologic oxidation. But depending upon whether this production of the hydrogen occurs in the presence or in the absence of oxygen, totally different effects (as an oxidation in the one case or a reduction in the other) may be brought about.

If to a fresh mixture of Fehling's solution and formaldehyde, or to one

in which partial reduction to metallic copper has already occurred, is added a reducible substance, it is found that, if oxygen is admitted to the reaction mixture, the reducible substance is not affected, while it is deoxidized if the oxygen is shut off. Such dyes as methylene blue or phenolsulphonephthalein which, with loss of oxygen yield colorless compounds, work best in this regard. Dyes which are easily distinguishable from the blue of Fehling's solution are naturally to be preferred. The red of phenolsulphonephthalein is, therefore, particularly good. If some of this dye is added to a mixture of Fehling's solution and formaldehyde (150 c.c. Fehling's solution plus 15 c.c. 40 per cent formaldehyde plus 0.6 gram phenolsulphonephthalein) and the resulting solution is divided, one-half being poured into a tall test tube in which it is but little exposed to oxygen, while the other half is kept in a flat dish with a large surface exposed to oxygen, it is observed that the red color of the phenolsulphonephthalein is lost after some hours in the tube protected against the air, while it remains unchanged in the open dish. The nascent hydrogen, in other words, unites in the latter instance with the oxygen of the air and leaves the dye unaffected, while in the absence of atmospheric oxygen the hydrogen seizes upon the oxygen of the phenolsulphonephthalein and decolorizes it.* The phenolsulphonephthalein can be shown to be still present in the tall test tube and in a readily oxidizable state. It is only necessary to shake the tube a little so as to get some of the atmospheric oxygen dissolved in the upper layers of liquid or to pour the whole reaction mixture into a second test tube through the air, to see the bright red color of the phenolsulphonephthalein reappear.

We see in these reactions an analogy to those upon which Hoppe-Seyler laid much stress and an illustration of the important differences so long recognized in biochemical reactions occurring in the presence of oxygen or in its absence. If we regard phenolsulphonephthalein as the analogue either of a protoplasmic "food" or of a protoplasmic "poison" to that "fermentation" mixture which we have likened to a biologic respiration system, it is obvious that the fate of the "food" or "poison" and hence of its effects upon the substrate, is something totally different in the presence and in the absence of oxygen. The same respiratory "ferments" produce effects in the one case which are totally lacking in the other.

*The behavior of the methylene blue is particularly interesting because it is the substance upon which Paul Ehrlich made his fundamental studies in tissue oxidation. Phenolsulphonephthalein is of interest because it is this dye which has found such extensive use in various functional tests for kidney efficiency. We have repeatedly emphasized (Martin H. Fischer, Oedema and Nephritis, New York, 1915, ed. 2, 621) the great care necessary in the interpretation of all functional tests, especially when such are used to demonstrate functional incapacity. In the specific case of the phenolsulphonephthalein test for kidney efficiency, for example, not only must it be remembered that a normal dye output can still be obtained, even when but as little as one-fourth to one-eighth the total kidney substance is still intact, but with any amount of normal kidney tissue various extrarenal factors in no way associated directly with the kidney state may make for a too low dye output even though the kidney itself is not primarily at fault. Anything which increases the adsorptive power of the body colloids for the phenolsulphonephthalein, for example, will work in this direction. In this way the dye is held in the body tissues, and, failing to get to the kidney, a low phthalein output results. E. B. Reemelin (Lancet-Clinic, 1916, cxv, 327) and Raphael Isaacs (Am. Jour. Physiol., 1916, xlii, 163) have shown that every increase in the acid content of the colloids of the blood—in other words, in all states in which there is an abnormal production or accumulation of acids in the body—will make this hold fast the injected dye and so it fails to come out in the kidney even when this organ is normal. But what is of especial interest in connection with the experiment detailed above is that it was devised to demonstrate in an ordinary physicochemic reaction mixture, the observation of E. C. Kendall (Am. Jour. Physiol., 1917, xlii, 592) that phenolsulphonephthalein is reduced to a colorless compound by tissue pulp whenever oxygen is absent, while this reduction fails to be made in its presence. Failure to excrete phenolsulphonephthalein may mean, therefore, not only a loss of kidney function to below the last quarter or eighth of its functional capacity, but a state of high general acid content, or an absence of oxygen, or these several factors together.

THE MASTIC TEST FOR THE DIAGNOSIS OF CEREBROSPINAL SYPHILIS

By Fletcher Langdon, M.D., Cincinnati, Ohio.

THE mastic test was published in 1915, by Emanuel, in an effort to find a simple and reliable test for the diagnosis of syphilis in the cerebrospinal fluid.

This test depends on the precipitation of a solution containing gum mastic, in cases of syphilis, and no change in the solution in negative cases.

The method in use was devised by J. A. Cutting* of the Agnew's State Hospital, Agnew, California, and is as follows: A stock mastic solution is made consisting of 10 grams of pure gum mastic in 100 c.c. of absolute alcohol; this is filtered and kept in tightly stoppered bottles in which it will keep indefinitely. One part of this stock solution is added to nine parts of absolute alcohol and the mixture is then insufflated into forty parts of distilled water, producing an opalescent solution which keeps well for several days and is the solution used in the actual test.

The absolute alcohol used in the test should be of highest purity and not merely dehydrated alcohol. Make a 1.25 per cent solution of sodium chloride (NaCl) in distilled water and to this add 1 c.c. of a 0.5 per cent solution of potassium carbonate for each 100 c.c. of NaCl solution used.

Six test tubes are placed in a rack; in the first tube is placed 0.5 c.c. of the spinal fluid, to be tested, and 1.5 c.c. of the combined 1.25 per cent NaCl and 0.5 per cent potassium carbonate; place in tubes Nos. 2, 3, 4, 5, and 6, one c.c. each of the 1.25 per cent NaCl and 0.5 per cent potassium carbonate solution. Remove, with a 1 c.c. bulb pipette, 1 c.c. of the mixture from the tube No. 1 and place in No. 2, and from No. 2 to No. 3, etc., until tube No. 5 is reached. The spinal fluid mixture (1 c.c.) is discarded from tube No. 5; tube No. 6 is the control tube and receives none of the spinal fluid mixture.

Mix the fluid in the test tubes by shaking or stirring with a clean glass rod. Add 1 c.c. of the mastic emulsion to each of the six tubes and mix again thoroughly. Allow to stand in a warm place (room temperature) for from twelve to eighteen hours, or incubate at 37° C. for six to twelve hours or incubate at 37° C. for two hours and centrifuge before reading.

In positive cases the mastic will be precipitated in the first one, two, three, or four tubes (or higher in strongly positive cases) leaving the supernatant fluid clear and the mastic a white, flocculent precipitate at the bottom of the tube, though in some cases the opalescent appearance of the fluid remains the same, with, however, a fine white precipitate of mastic at the bottom of the tube. The control should never change its appearance.

In the serologic laboratory of the Cincinnati General Hospital we tested by this method 101 specimens of cerebrospinal fluid. These specimens were, whenever possible, submitted also to the Wassermann, gold curve, globulin (butyric acid test) and a blood Wassermann test.

The following table shows what results were obtained. These 101 speci-

*Jour. Am. Med. Assn., June 16, 1917.

mens were not selected, but came to the laboratory for the routine serologic examination (Table I).

TABLE I*

CEREBROSPINAL FLUID		MASTIC			GOLD CURVE				GLOBULIN		BLOOD WASS.				
		Positive	Negative	Doubtful	Paretic	Luetic	Meningitic	Negative	Positive	Negative	Positive	Negative	No Gold Curve	No Globulin	No Blood Wassermann
Positive Wassermann	31	26	3	2	21	4	2	1	26	2	13	2	3	3	16
Negative Wassermann	62	6	54	2	3	13	1	39	4	52	0	9	6	6	53
Doubtful Wassermann	8	1	7	0	0	3	0	5	3	5	1	2	11	2	0
Gold curve paretic	22	16	6	0	0	0	0	0	23	1	11	2	0	0	0
Gold curve meningitic	3	2	1	0	0	0	0	0	3	0	0	0	0	0	0
Gold curve luetic	18	8	9	1	0	0	0	0	15	5	2	1	0	0	0
Gold curve negative	49	2	46	1	0	0	0	0	1	14	1	10	0	0	0
Globulin negative	51	2	50	1	1	5	0	47	0	0	1	10	0	0	0
Globulin positive	41	29	11	1	23	15	3	1	0	0	13	3	0	0	0

*Owing to the fact that a number of specimens of cerebrospinal fluid contained blood, it was impossible to make the gold curve and globulin tests in every case. It was also impossible to obtain specimens for a blood Wassermann in over half of the cases.

After completing these 101 mastic tests in connection with the other routine serologic tests, our conclusions were:

1. That the mastic test, in our hands, is probably not as delicate or reliable as Lange's colloidal gold test (gold curve) properly performed.

2. That the gold curve, properly performed, will indicate whether the syphilis in the cerebrospinal fluid is of the paretic, luetic, or meningitic type, while the mastic test merely shows a positive or negative result.

3. That the colloidal gold solution is but little more difficult to make than the mastic solution when one uses care to obtain a high-grade chloride of gold for use, and that the colloidal gold solution may be made in comparatively large quantities and will keep until used.

4. That, while the gold curve requires twice the number of test tubes for each test and also double distilled water, the mastic test requires pure absolute alcohol (expensive and difficult to obtain at present) and that the mastic glassware is much more difficult to clean than the gold curve glassware and it is of primary importance that all glassware used in either test be absolutely clean, the gold curve glassware being cleaned with soap and water, then with sulphuric acid, then rinsed in distilled water and dried. The mastic ware must be cleaned with soap and water, then dried and thoroughly rinsed in strong alcohol to remove the mastic adhering to the glass.

I am under great obligations, in this work, to Dr. Oscar Berghausen, in charge of the serologic laboratory of the Cincinnati General Hospital (where this work was done) and to Mrs. Mary Rivers, the assistant demonstrator in serology under the Moos fund, and to Dr. Foy Payne for much help and kindness during the course of this work.

AN IMPROVED METHOD FOR ANESTHETIZING ANIMALS*

By John A. Higgins, St. Louis, Mo.

A METHOD for administering ether to animals, which I have seen quite often in the laboratory, is described by Jackson.† This method may be satisfactory if properly executed, but the following method seems much safer and can easily be handled by one man.

The apparatus used in this method (see figure) consists of an empty one-pound ether can cut in halves, of which the upper half is here used. The front end of the ether can *(A)* is perforated with numerous holes and allows perfect ventilation. Around the lower, or open, edge of the can (*B*) are also perforated numerous holes, to which is fastened much cotton covered with gauze, allowing only a sufficient opening for the average dog's nose and mouth to enter. On the top of the ether can (*C*) is a cup-shaped piece of metal in which ether is poured and allowed to enter the inside of the ether can. On each side of the ether can (*D*) are fastened wire lugs to which are fastened short stout straps and buckles.

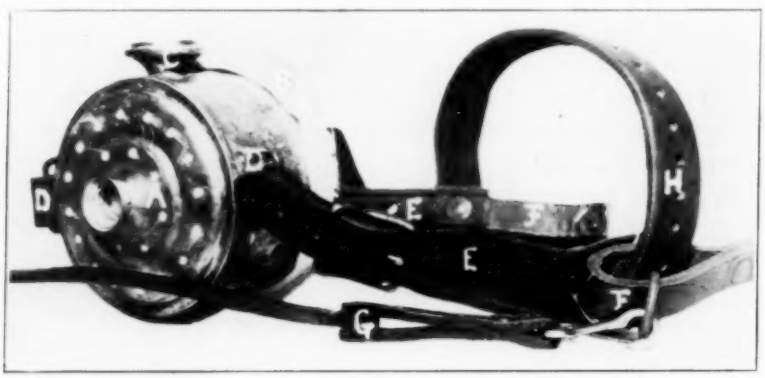

Fig. 1.

This section just described is used in the fashion of a muzzle and is held in position on the animal by straps *E* which in turn, are riveted onto the inside of straps *F*. Straps *F* are stout, about three inches long, and both ends are riveted together after placing about an inch in diameter ring on each strap. Strap *H* is an ordinary stout collar strap placed around the neck of a dog just tight enough to prevent the dog from slipping his head through the collar.

On the far ring at strap *F* is fastened a strap long enough to go around a solid stationary block or post, and fastened onto the ring on the near side at strap *F*. A snap catch may here be used as illustrated. Previous to use, the ether can should be lined with a layer of cotton.

After arranging this apparatus on a dog, the dog is set in a resting erect position on the floor. Grasp the dog's forepaws with the left hand, with the

*From the Department of Pharmacology, Washington University Medical School, St. Louis.
†D. E. Jackson: Experimental Pharmacology, C. V. Mosby Co., 1917, p. 77.

right knee pressing down on the dog's back to keep him in place. The ether is then poured in cup *C* with the anesthetist's right hand. When the dog is well anesthetized, remove the apparatus by loosening strap *H*, place the dog on the operating table and proceed as usual.

Where a very strong dog is to be anesthetized it might be advisable to use, in connection with the previously described apparatus, a board platform set on the floor and large enough for the operator and dog to stand upon. On the rear end of the board platform should be fastened two strong upright pieces to which the dog's hind quarters are securely fastened. The dog's hind quarters should be fastened just above the middle joint of the legs and while he is in a standing position. Where the platform procedure is used, it will eliminate holding the dog in position with the right knee as previously described. This last procedure of holding a dog could be used to great advantage in the laboratory where hypodermic injections of drugs or serums, as in sensitizing anaphylactic dogs, are required.

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EDITORIALS

The Nervous Mechanism in Thyroid Secretion

SEVERAL years ago Professor Asher, of the University of Bern, demonstrated an experiment which was interpreted as proving the existence of a nervous mechanism to the thyroid glands. The thyroidal nerves were stimulated with induced electric shocks of low intensity, and the response of the heart to subminimal vagus stimulation was determined at stated intervals during the experiment. If the stimulus became effective in slowing or inhibiting the heartbeat, it was taken as proof that the thyroid had produced an extra amount of thyroidal hormone which rendered the nerve endings of the vagus hypersensitive. Here, then, was experimental proof that the secretion of the thyroid gland exalts the excitability of nerves, and that the thyroid secretion is determined by a nervous mechanism. The surgeon found in these results a reason for the removal of the glands in Graves' disease, where there is marked nervous excitability.

Unfortunately the results of many physiologic experiments are not sufficiently well defined to satisfy the critic, and Asher's work was not widely accepted by physiologists.

Some years later Cannon[1, 2] and his pupils at Harvard produced apparently direct evidence for the existence of secretory nerves to the thyroid. One method they employed for showing a nervous mechanism in thyroid excretion in cats consisted in fusing the anterior root of the phrenic nerve with the cervical sympathetic, and noting its effect on the thyroid function. These workers state that symptoms resembling those of Graves' disease in man developed. There were marked tachycardia, loose movements of the bowels and falling of the hair. The animals were unusually excitable. The basal metabolism was very markedly increased. The pupil was larger on the operated side, and in one of the animals exophthalmus and respiratory hippus developed on the operated side. They found that in one animal the removal of one of the glands stopped the progress of the disease, and the cat lived for seven months, when it was purposely killed. The other animals lived less than three months after the onset of the symptoms.

Troell[3] and Burget[4] have repeated the experiments of Cannon with negative results, and Marine, Rogoff, and Stewart[5] have just published the results of experiments on ten cats in which the operation made by Cannon was repeated. These observers failed to obtain the results reported by Cannon and his pupils in any of their series of experiments. In animals where there can be no doubt that the anastomosis was successful, they failed to find any evidence of impulses coming from the respiratory center as shown by a rhythmic change of the iris synchronous with the respiration. Exophthalmus and loss of weight were not present, nor was there any evidence of thyroid enlargement.

Such contrary results coming from men of recognized research ability are hard to understand. A significant and important clinical observation, bearing on the relationship of nerve strain and the incidence of Graves' disease was reported to the editor by Hoover, late Major in the United States Medical Reserve Corps. He states that, so far as he observed and could ascertain, while on duty in France, no cases of Graves' disease were found among the soldiers which could be attributable to the strain of the life incident to active warfare. Certainly if Graves' disease is the result of nerve strain, as some claim, it should be present in a large number of cases at the fighting front.

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—*R. G. P.*

Volunteer Medical Service Corps

FOR the purpose of completing the mobilization of the entire medical and surgical resources of the country, the Council of National Defense has authorized and directed the organization of a "Volunteer Medical Service Corps," which is aimed to enlist in the general war-winning program all reputable physicians and surgeons who are not eligible to membership in the Medical Officers' Reserve Corps.

It has been recognized always that the medical profession is made up of men whose patriotism is unquestioned and who are eager to serve their country in every way. Slight physical infirmities or the fact that one is beyond the age limit, fifty-five years, or the fact that one is needed for essential public or institutional service, while precluding active work in camp or field or hospital in the war zone, should not prevent these patriotic physicians from close relation with government needs at this time.

It was in Philadelphia that the idea of such an organization was first put forward, Dr. William Duffield Robinson having initiated the movement resulting in the formation last summer of the Senior Military Medical Association with Dr. W. W. Keen as president—a society which now has 271 members.

Through the Committee on States Activities of the General Medical Board the matter of forming such a nationwide organization was taken up last October in Chicago at a meeting attended by delegates from forty-six states and the District of Columbia. This Committee, of which Dr. Edward Martin and Dr. John D. McLean—both Philadelphians—are respectively chairman and secretary, unanimously endorsed the project. A smaller committee, with Dr. Edward P. Davis, of Philadelphia, as chairman, was appointed to draft conditions of membership, the General Medical Board unanimously endorsed the Committee's report, the Executive Committee—including Surgeons-General Gorgas of the Army, Braisted of the Navy, and Blue of the Public Health Service—heartily approved and passed it to the Council of National Defense for final action, and the machinery of the new body has been started by the sending of a letter to the state and county committees urging interest and the enrollment of eligible physicians.

It is intended that this new corps shall be an instrument able directly to meet such civil and military needs as are not already provided for. The General Medical Board holds it as axiomatic that the health of the people at home must be maintained as efficiently as in times of peace. The medical service in hospitals, medical colleges and laboratories must be up to standard; the demands incident to examination of drafted soldiers, including the reclamation of men rejected because of comparatively slight physical defects; the need of conserving the health of the families and dependents of enlisted men and the preservation of sanitary conditions—all these needs must be fully met in time of war as in time of peace. They must be met in spite of the great and unusual depletion of medical talent due to the demands of field and hospital service.

In fact, and in view of the prospective losses in men with which every community is confronted, the General Medical Board believes that the needs at home should be even better met now than ever. The carrying of this double burden will fall heavily upon the physicians, but the medical fraternity is confident that it will acquit itself fully in this regard, its members accepting the tremendous responsibility in the highest spirit of patriotism. It will mean, doubtless, that much service must be gratuitous, but the medical men can be relied upon to do their share of giving freely, and it is certain that inability to pay a fee will never deny needy persons the attention required.

It is proposed that the services rendered by the Volunteer Medical Service Corps shall be in response to a request from the Surgeon-General of the Army, the Surgeon-General of the Navy, the Surgeon-General of the Public Health

Service, or other duly authorized departments or associations, the general administration of the corps to be vested in a Central Governing Board, which is to be a committee of the General Medical Board of the Council of National Defense. The State Committee of the Medical Section of the Council of National Defense constitutes the governing board in each state.

Conditions of membership are not onerous and are such as any qualified practitioner can readily meet. It is proposed that physicians intending to join shall apply by letter to the Secretary of the Central Governing Board, who will send the applicant a printed form, the filling out of which will permit ready classification according to training and experience. The name and data of applicants will be submitted to an Executive Committee of the State Governing Board, and the final acceptance to membership will be by the national governing body. An appropriate button or badge is to be adopted as official insignia.

The General Medical Board of the Council of National Defense is confident that there will be ready response from the physicians of the country. The Executive Committee of the General Medical Board comprises: Dr. Franklin Martin, Chairman; Dr. F. F. Simpson, Vice-chairman; Dr. William F. Snow, Secretary; Surgeon-General Gorgas, U. S. A.; Surgeon-General Braisted, U. S. Navy; Surgeon-General Rupert Blue, Public Health Service; Dr. Cary T. Grayson; Dr. Charles H. Mayo; Dr. Victor C. Vaughan; Dr. William H. Welch.

Immunity to Tuberculosis

"ONLY a tuberculous animal is immune to tuberculosis." This statement which would appear a paradox is yet true. It has been proved that one tubercle focus within an animal renders that animal immune to moderate reinfection from without. Should the focus be completely removed, this comparative immunity is also completely removed.

The animal with a focus of tuberculosis can in time, however, be overwhelmed by bacilli escaping from such an area should the encapsulation yield to destructive influence from within and at times probably to forces from without.

A safely encapsulated tuberculous focus is probably present in the bodies of most human beings. Such safe infection, the result of accident and of bodily resistance, protects the majority of mankind against active disease, yet Nature, in accomplishing such infection, and "vaccination" in the many, destroys a large number of our race. The evidence of autopsies corroborates what is learned through tuberculin tests that large numbers of people contain in their bodies such foci of tubercles. In possibly a majority of cases these foci are best found in lymph nodes, and often many of the tubercle bacilli found in these nodes prove to be dead.

Success in medical investigation is usually attained by imitating as closely as possible the processes of Nature. We triumph over diphtheria by adding to Nature's efforts quantities of antitoxin. We can easily conceive that Pasteur's employment of attenuated vaccines, the employment of dead bacteria so suc-

cessful in typhoid immunization, some of the accidents of bacterial dosage, may be now and then Nature's methods. Can we imitate Nature at her best in the production of a focus of tubercles in an animal, without the danger of following her at her worst—the destruction of the animal through tuberculosis?

A step in such a direction is possibly suggested in experiments, referred to by Krause[1] as simple and ingenious, a preliminary report of which has recently been published by Webb, Ryder, and Gilbert.[2] These workers have demonstrated that tuberculous lymph nodes can be transplanted into normal guinea pigs and that immunity, similar to that of a tuberculous animal will result. In time, however, such an immunized animal was found to succumb, through the escape of tubercle bacilli from within the transplanted focus. The attempts to provoke a stronger encapsulation of the lymph node by the employment of irritants also lacked success.

The results of surrounding these infected lymph glands with collodion, fish skin, etc., will be looked forward to with interest. Such "encapsulation" would probably allow immune processes resulting from the conflict of bacilli and lymphoid structures, to permeate into the host, yet should inhibit easily a spread of the bacilli. It will be important to determine the length of life of these bacilli, and also that of the tissue cells; these we understand are being investigated.

Heymans,[3] of Belgium, has reported several times that some immunity could be procured in animals by planting within them tubercle bacilli cultures which were enclosed in sacks made of the marrow membrane of the reed cane. Such a procedure of culture *in vivo* was first suggested by Metchnikoff. Heymans employed broth, exudates, and in some series, polymorphonuclear exudates for the culture media in these sacks. In general the bacilli were found to increase and multiply. Such sacks suspended in tubes of broth media were found to transmit tuberculin qualities to this media. Heymans reports in some experiments little if any immunity was produced in the animals receiving these sacks, and again in other experiments he reports some successes, not only in the production of some immunity to infection, but also as a useful procedure for the treatment of infected animals.

Djounskowsky is quoted as stating that toxins from the retained bacilli would diffuse through such an osmotic membrane. In diphtheria work a collodion membrane is employed to separate toxins from antitoxins, toxins diffusing through the membrane.

Jobling[4] has stated that unless a bacterial organism is tryptogenic a nourishment containing amino acids or peptones must be supplied. Such foodstuffs will pass through a collodion membrane.

That tubercle bacilli enclosed in collodion or other sacks will survive many months we know from the work of Heymans, who found virulent bacilli after eight months; and from the experiments of A. S. Griffith[5] of the British Royal Commission on Tuberculosis. This investigator attempted to change the human type of tubercle bacillus into the avian type by placing collodion capsules containing human tubercle bacilli within the peritoneal cavities of fowls and pigeons. The bacilli in some instances were found to be alive after more than two years.

Heymans' results suggest that tuberculin products were alone diffused, and

that the possible slight immunity demonstrated might be dependent on these. Products resulting from a conflict between bacilli and cells were not present.

It is recognized that in immunity to tuberculosis we are dealing with humoral as well as cellular qualities. The mechanism is probably very complex.

Much and Leschke[6] employed extracts of highly immunized animals and of tuberculous animals. These extracts were prepared from different organs and even from whole guinea pigs. Their conclusion was that the inoculation of such extracts failed to transmit any cellular immunity. Much, the advocate of partial antibodies in the search for humoral immunity, states that even with this determined no end is reached because cellular immunity must also be investigated.

It must be evident as the result of such vast experimentation in tuberculosis that the continued conflict of living bacillus and of living cell produces some product essential to the production of immunity.

The transplantation of a tuberculous lymph node or a piece of tuberculous liver is a new idea in experimental work for the production of immunity and opens up large possibilities for investigations.

Can the vital conflict, represented in such a transplant, be kept safely isolated without injury to the transplant and without injury to the host, a method of protection against tuberculosis will have been found.

The publication of this preliminary report will allow many laboratories to engage in this research.

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—*G. B. W.*

Lymphocyte Elements and Tuberculosis

A VAST amount of research work has been published which was undertaken to prove that the lymphocyte elements play a very important part in the defense of an organism against the bacillus of tuberculosis. As Krause[1] correctly states, nothing in this regard is conclusively proved, and we must grant that any evidence we have is possibly only circumstantial.

That any minute foreign substance may be taken to a lymph node or be taken up perhaps by a phagocyte we know, yet when we witness a lymph node arresting a tubercle bacillus or a polymorphonuclear leucocyte engulfing a gonococcus—with at times a resulting destruction of these elements and a triumph for the infecting organism—we believe that usually a battle has been fought.

Now, it is true that any tissue of the body may at times be attacked by the bacillus of tuberculosis and triumphantly defend itself—one tissue better than another—from such attack.

Much research work has proved that the formation of a tubercle in any

vulnerable tissue is a defensive mechanism of nature. Of what, then, is such a tubercle composed? It is composed of the giant cells, the so-called epithelioid cells, and the zone of lymphoid or mononuclear cells.

Whatever tissue, therefore, that defends itself against the bacillus of tuberculosis appears to receive the help of the lymphoid cells. Exudates from tuberculous processes also characteristically contain lymphocytes and the contents of cold abscesses suggest the triumphant bacilli may "enslave" the lymphocyte ferments. It is not necessary to inquire here into the origin of the giant and epithelioid cells.

The mysteries of natural processes are often difficult to interpret, yet many such processes are in time easily and reasonably explained. We can hardly conceive that Nature would send the lymphocyte cell into what we regard as a possible conflict unless she needed it there.

It is true that at times, especially in "immune" animals, a tubercle may appear to be formed and even absorbed without the presence of lymph cells being noted in corresponding specimens. Yet is it completely proved that not even a few are present possibly very early in the process? The qualities discovered in and attributed to the lymphocyte cell have been related here[2] before, so it is not necessary to repeat them. That immunity to tuberculosis is accompanied by a marked lymphocytosis, or that lowered resistance is contingent on lymphopenia are expectations possibly not proved, yet some investigations appear to decidedly support them.

That important immune and possibly specific processes are provided by the lymphocyte cells we must continue to suspect by virtue of the constant presence of such elements—possibly only as "infantry"—in the conflict of tissues and tubercle bacilli. The lymph nodes themselves must, therefore, also be judged as defensive as well as mechanical hindrances to the development of tuberculosis.

"Natura non facit inutile aut superflua."

BIBLIOGRAPHY

[1]Krause, A. K.: Am. Rev. Tuberc., Feb., 1918.
[2]Webb, G. B.: Immunity in Tuberculosis, Jour. Lab. and Clin. Medicine, 1916, i, 414.

—*G. B. W.*